The Urinary 1-hydroxypyrene Biomarker in Street Janitors Exposed to Traffic Related Benzo[a]Pyrene During One Islamic Calendar Year in Madinah, Saudi Arabia

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ABSTRACT

Background

Madinah is the second most important Muslim holy city in Saudi Arabia. Therefore, during the holy months, traffic congestion and consequent air emission of several pollutant compounds, mainly carcinogenic Benzo[a]pyrene (BaP), are expected to reach high levels in certain places. The emission of these pollutants into the atmosphere increases their concentration, which in turn increases the risk of exposure through inhalation. Inhalation of air BaP is the main route of entry into the human body, and so the aim of the present study was to assess exposure to air BaP through urinary 1-hydroxypyrene (1-OHP) in street janitors. The study also aimed at finding the relation between this biomarker and daily exposure to air BaP with traffic volume and meteorological factors, such as wind speed, temperature, wind direction, relative humidity, and precipitation.

Methods

The study was performed during one Islamic calendar year from 1/4/1438 – 30/3/1439 (December 2016 to November 2017) in Madinah City. The research was designed to include three traffic locations with high traffic density and two control locations with low traffic activity. In these locations, BaP was measured daily in the breathing-zone air of the street janitors using $PM_{2.5}$ personal samplers, and in the air of the five studied locations using high-volume air samplers. Daily measures including traffic volume (cars, medium and heavy vehicles), temperature, humidity, wind speed, and precipitation, were recorded at the five studied locations. 20 male participants (street janitors) aged between 25 and 35 years were recruited in the study from each of the five studied locations. In total, 100 street janitors were involved in the study. Two urine samples were obtained from each participant after their work shift and in the early morning of the next day to measure 1-OHP. Summary statistics, including medians, were used to describe the data. Graphs, including

calendar and time series plots were used to visually present the data. Data were analysed using methods that included Spearman's rank correlation, Mann-Whitney, and Kruskal-Wallis tests.

Results

It was found that BaP and 1-OHP concentrations were significantly higher in the heavy traffic locations compared to the control locations. The research also found that BaP and 1-OHP concentrations were significantly higher during the holy months of the Islamic calendar than the normal months. For example, the median concentrations of 1-OHP in traffic locations during the holy months of Ramadan, Dhu al-Qadah, Dhu al-Ḥijjah and Muḥarram were 0.98, 0.99, 1.01 and 0.82 µmol/mol, respectively. The median concentrations of 1-OHP in normal months ranged between 0.71–0.74 µmol/mol. The median ambient BaP concentration during the holy months ranged between 0.59–0.63 ng/m^3, while in the normal months it ranged between 0.36–0.39 ng/m^3. It was also found that morning 1-OHP concentration was higher than after work 1-OHP concentration. The research also found that ambient BaP concentration was generally higher than the individual BaP concentration. Correlation results indicated that ambient BaP is positively correlated with CO ($r = 0.59$, $p<0.001$) and traffic volume ($r = 0.74$, $p<0.001$. It was also found that individual BaP is positively correlated with CO ($r = 0.56$, $p<0.001$) and traffic volume ($r = 0.85$, $p<0.001$). Meteorological conditions such as temperature, relative humidity and precipitation were found to have no effect on the concentration of both ambient and individual BaP.

Conclusion

The research found that the higher the traffic volume in Madinah, especially during the holy months, the higher the ambient BaP pollutants, and the higher the chance of people being exposed to BaP pollutants (individual BaP). Therefore, it is recommended that efforts to reduce the number of vehicles in Madinah be adopted. Two approaches are suggested: enacting laws preventing traffic, tax law in traffic areas to reduce the number of cars in the traffic areas, and encouraging the use of hybrid buses, electric trains and cars in Madinah. It is, however, important to note that the higher correlations found in this study were likely due to the controlled environment.

DEDICATION

This piece of work is dedicated to:

My parents

My wife and my daughters

My sisters & brothers

With my sincere thanks for your love, patience and support.

ACKNOWLEDGEMENTS

My journey of becoming a PhD holder began when I was admitted as a PhD student at Newcastle University. The journey towards attaining this degree has not been without challenges, and it could not have been successful without the help as well as support of a number of people. It is for this reason that I would like to thank those who contributed towards the success of the PhD degree.

First, and in a special way, I would like to show my deepest appreciation to my supervisors, Dr Richard McNally and Dr Anil Namdeo, for their dedication, time, scholarly comments, attention and invaluable advice and guidance throughout my PhD programme. Their constructive critique as well as our academic discussions have been precious to me. In particular, Dr Richard McNally advised me on every aspect of my study. He was always there for me, and never showed me any signs of being tired of my problems, requests, and questions. In a special way, I would also like to thank Dr Anil Namdeo for giving me support and direction, as well as for his insights and invaluable guidance and advice, which have helped me to reach this important milestone. It is without doubt that, if not for their limitless patience, dedication, time and encouragement, accomplishing this PhD would not have been possible. I am grateful for the opportunity to have been a student with this team. I also cannot fail to mention Prof. Elaine McColl for her support and valuable advice, which enabled me to make it through some difficult times during my study.

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I do not forget to thank the Madinah Regional Municipality for their support and allowing me to access their laboratory, the environmental data on air pollution, and for facilitating access to the participants in the study. I also would like to thank the Healthy Cities Programme in Madinah. In addition, I thank the General Authority of Meteorology and Environmental Protection in Madinah for providing daily weather data.

Other thanks should go to the Saudi Ministry of Education and the Saudi Cultural Bureau in London for sponsoring and funding my PhD study in Newcastle University.

Last, but not least, I honourably wish to thank my beloved parents, who constantly gave me their support me through their best wishes and sincere prayers. On a special note, I am also very grateful to my sisters, brothers, nephews and nieces, for their continuous encouragement, prayers and best wishes. I would also like to express my appreciation to Saudi Society friends in Newcastle, who also helped me along the way.

Finally, I owe my faithful wife, Wedyan, and my lovely daughters, Tala and Dana, exceptional thanks and gratitude for their patience, love and endless support that has enabled me to complete my studies successfully.

STATEMENT OF CONTRIBUTION

This is to declare that the work contained in this thesis comprises original work conducted by the student under the supervision of Dr Richard McNally and Dr Anil Namdeo. This thesis has not been submitted for the award of any other degree at any other institution.

CONFERENCES AND PUBLICATIONS

1. Khalid Kordi, Dr. Richard McNally & Dr. Anil Namdeo. **Biological and chemical monitoring of occupational exposure to carcinogenic compound (Benzo[a]pyrene) due to vehicle traffic in Madinah, Saudi Arabia.** The Third Annual Newcastle University Postgraduate Cancer Conference, 16th March 2018, Great North Museum: Hancock. (Appendix 7.8).

2. Khalid Kordi, Dr. Richard McNally & Dr. Anil Namdeo. **The Urinary 1-hydroxypyrene Biomarker in Street Janitors exposed to Air Quality Changes Associated with Religious events in Madinah, Saudi Arabia.** The IHS Annual PGR, Newcastle University, 5th June 2018, the Research Beehive. (Appendix 7.7).

3. Khalid Kordi, Dr. Richard McNally & Dr. Anil Namdeo. **The Urinary 1-hydroxypyrene Biomarker in Street Janitors exposed to Air Quality Changes Associated with Religious events in Madinah, Saudi Arabia.** International Conference on Transport & Health (ICTH) 24–27 June 2018, Mackinac Island, USA). (Appendix 7.7).

4. Kordi, K., McNally, R. and Namdeo, A., 2018. **The Urinary 1-Hydroxypyrene Biomarker in Street Janitors Exposed to Air Quality Changes Associated with Religious Events in Madinah, Saudi Arabia.** Journal of Transport & Health, 9, pp. S52-S53. (Appendix 7.9).

My posters, presentations and publications are shown in the Appendix.

ACRONYMS

1-OHP	Urinary 1-hydroxypyrene
BaP	Benzo(a)pyrene
CO	Carbon monoxide
DNA	Deoxyribonucleic acid
FD	Fluorescence Detection
GCMS	Gas Chromatography –Mass Spectrometry
HCs	Hydrocarbons
HPLC/MS	High Performance Liquid Chromatography – Mass Spectrometry
LC-MS	Liquid Chromatography – Mass Spectrometry
NIOSH	The National Institute for Occupational Safety and Health
NOx	Nitrogen Oxides
PAHs	Polycyclic Aromatic Hydrocarbons
PHE	Public Health England
PM	Particulate Matter
PPM	Parts Per Million
REL	Recommended Exposure Limit
SFS	Synchronous Fluorescence Spectrometry
SVOCs	Semi-volatile Organic Compounds
VOCs	Volatile Organic Compounds
WHO	World Health Organization

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

Chapter 1: Introduction

1.1. Introduction

Traffic is globally considered one of the most critical sources of air pollution in urban areas, and vehicular emissions are one of the major sources of this kind of pollution. Such pollutants may cause a number of health problems, including: cardiovascular disease, lung cancer, respiratory disturbances and asthma (Lim *et al.*, 2005b; Patel *et al.*, 2010b; Katoshevski *et al.*, 2011). Diesel, petrol and other forms of fossil fuel, such as liquified petroleum gas (LPG), used to power transportation tend to release a number of toxic substances with pro-oxidant properties, including oxides of nitrogen (NOx), CO and $PM_{2.5}$. Such harmful substances include, but are not limited to, heavy metals, polycyclic aromatic hydrocarbons (PAHs), and volatile organic compounds (VOCs) (Krzyżanowski *et al.*, 2005; WHO, 2005).

PAHs are mainly introduced into the atmosphere by the combustion process, and combustion in diesel engines is one of the most significant sources of PAHs in the urban environment (Li *et al.*, 2008). PAHs are normally emitted by diesel engines mainly because of the presence of incomplete combustion, resulting in the presence of unburned fuel (Unwin *et al.*, 2006). This type of source is known as petrogenic (Li *et al.*, 2008). PAHs may also be formed during the process of the combustion, especially if the ideal conditions of fuel combustion are not present (Kim *et al.*, 1999; Zhang *et al.*, 2006; Hu *et al.*, 2007a; Alkurdi *et al.*, 2013). Benzo[a]pyrene (BaP) is considered one of the most critical PAHs and is usually produced from incomplete combustion of diesel and petrol, as well as other fossil fuels (Kim *et al.*, 1999; Zhang *et al.*, 2006; Hu *et al.*, 2007a; Alkurdi *et al.*, 2013).

Since urban areas tend to be associated with a higher traffic volume than rural areas, vehicular emissions are the main sources of BaP in these areas (Barakat, 2002). They tend to be persistent and ubiquitous contaminants, and have carcinogenic, endocrine disrupting, and mutagenic effects on both wildlife and humans (Unwin *et al.*, 2006).

Usually, people are exposed to BaP through ingestion, inhalation, and contact through skin (IARC, 2002). The toxicity of PAHs is often associated with their ability to undergo a biotransformation process, in which they are converted to reactive metabolites (Lodovici and Bigagli, 2011). As a result, exposure to BaP has been associated with diabetes mellitus (Alshaarawy *et al.*, 2014; Yang *et al.*, 2014), cardiovascular conditions (Xu *et al.*, 2010; Feng *et al.*, 2014), and metabolic syndrome (Brocato *et al.*, 2014; Hu *et al.*, 2015b).

Due to the carcinogenic properties, BaP has attracted the attention of Kim *et al.* (1999), particularly as these pollutants penetrate the body through the lungs and skin (Unwin *et al.*, 2006). The exposure to BaP is usually monitored by measuring the concentration of 1-hydroxypyrene (1-OHP), which is a metabolite of pyrene in urine (Jongeneelen, 2001; Shin *et al.*, 2011; Savitz *et al.*, 2012). As a result, it is often used as a biomarker for exposure to BaP (Li *et al.*, 2008). Studies have shown a positive relationship between the respective concentrations of 1-OHP and BaP in air pyrene (Jongeneelen, 2001; Shin *et al.*, 2011; Savitz *et al.*, 2012).

Although there has been much monitoring of the level of BaP in different parts of the world, the Middle East has been far behind in this endeavour (Alghamdi *et al.*, 2015e). A case of concern is Saudi Arabia, where its industrial development has been a leading cause of toxic pollution (Magram, 2009). Madinah is one of the main cities in Saudi Arabia and is located in the western part of the county. It is considered to be one of the most important Muslim holy cities because it contains the mosque of the Prophet Mohammed. It's population is already approximately 2m (Brinkhoff, 2019). With the passage of time, the city has prospered and the population is increasing noticeably (Bazzaz *et al.*, 2011). Consequently, it is becoming crowded with people and vehicles, as the number of annual pilgrims is about 5m (Hajj, 2019).

For instance, the current population growth rate in the city is greater than 2.0% (Alshehry and Belloumi, 2015). Additionally, consumption of energy generated from fossil fuel (diesel, petrol and natural gas) is heavily subsidised in Saudi Arabia, making the cost of energy (especially diesel, petrol and natural gas) in Madinah among the lowest in the world (Alshehry and Belloumi, 2015). This has led to the overuse and misallocation of natural gas and oil resources, and subsidisation has reduced the incentive in the country's fast growing population to conserve energy in their various economic activities (Alshehry and Belloumi, 2015). For instance as of 2010, the GDP per unit of energy used was approximately PPP US$ 3.688 per kg of oil equivalent, while the energy use per capita was approximately 6,168 kg of oil equivalent, which was the highest in the entire world (Alshehry and Belloumi, 2015). The global avarege in 2010 was 1,851 kg of oil equivalent (Alshehry and Belloumi, 2015).

People are mainly reliant on vehicles for their transport, making cars the primary source of air pollution in the city, as fuel is generally inexpensive in Saudi Arabia (Al-Sheikh, 2013). It is also important to note that hybrid cars, as well as full electric cars, are generally uncommon in Saudi Arabia, making petrol and diesel powered vehicles the choice of transport in Madinah (Razeen, 2015). Since Madinah has become one of the major cities in the Kingdom of Saudi Arabia for economic, religious, cultural, and tourism reasons (Al-Sheikh, 2013), these factors have led to the establishment of a new road network, bridges, and tunnels to cope with the many cars.

1.2. Study Overview

This study was carried out in Madinah city, Saudi Arabia, using the available facilities in Madinah Municipal Laboratory over one year, as is described later in Chapter 3 (Sections 3.2 to 3.7). Five locations were selected to conduct this study, three of which were located at high traffic density locations, and two were considered as background locations which were not influenced by traffic density. BaP was measured by using two methods, which include a high-volume air sampler and personal air samplers. This was done to gain insight into the variability of BaP concentrations throughout the study period (one Islamic year), taking into account the most influential factors such as traffic volume, religious events and meteorological measurements. In addition, urine samples were collected from the participants (street janitors) who worked within the study locations. Their urinary 1-OHP were then measured to be used as a biomarker of exposure to BaP. All the data were statistically analyzed to explore the relationship between concentrations of BaP and the emergence of urinary 1-OHP signs in the urine samples.

1.3. Points of Strength

1. The Madinah Regional Municipality supported this PhD study by offering unlimited use of its advanced laboratories (with ISO 17025 certificate), air quality monitoring equipments, manpower and required data (Appendix 7.1).
2. The Madinah Municipality has a contract for sanitation and hygiene, employing 3,000 workers to patrol the streets of the city during their 8 hours of work per days. This research focused on this group of people as they are exposed to ambient air pollution directly. In addition, the fact that the Madinah Municipality provides them with meals, health care, transportation, and special workers’ accommodation helped the research in terms of controlling their diet, health status and smoking status.

1.4. Map and Location of Madinah in Saudi Arabia

Figure 1: Location of Madinah (violet square box) and three other major cities in Saudi Arabia (Assirey and El-Shahawi, 2015).

1.5. Hypotheses

Certain occupations such as driving, road construction and working in steel production plants have been associated with exposure to PAHs. For instance, Xu *et al.* (2018) and Mcclean et al. (2012a) found that conventional paving workers and crumb rubber modified asphalt workers exhibited higher levels of PHA metabolites in their urine, indicating exposure to the PAHs. Stec *et al.* (2018) found that firefighters are exposed to environments which involve burning items with various properties, and as a result they are exposed to PAHs. Hansen et al. (2004) found that bus drivers and mail carriers in Danish cities and urban areas have higher concentrations of PAHs in their system compared to the general population. The current study contributes to the literature by aiming at the more refined aspects of causes of elevated 1-OHP in a particular sub-group of the population (steet janitors), and to identify the key drivers. In this regard, since street janitors in Madinah work near traffic conjested areas, there is a chance that they are exposed to PAHs. The study hypothesises that:

Hypothesis 1: Exposure to traffic exhaust related Benzo[a]Pyrene will increase the concentration of urinary 1-OHP in humans.

Hypothesis 2: Traffic volume is positively correlated with ambient and individual BaP concentrations in Madinah.

Hypothesis 3: Ambient and individual BaP concentrations are positively correlated with CO concentration.

Hypothesis 4: Ambient and individual BaP concentrations are positively correlated with meteorological factors, such as temperature, relative humidity, precipitation and wind speed.

1.6. Aim of the Study

The aim of this study is to explore the effect of exposure to traffic-related BaP during one Islamic calendar year on street janitors in Madinah by quantifying the levels of their urinary 1-OHP.

1.7. Objectives

1. To identify the hotspots of BaP concentrations in Madinah and compare these with the background sites (control location).
2. To identify the major variables (i.e. traffic density, religious events, meteorological variations) that could affect BaP concentrations during one Islamic calendar year.
3. To quantify urinary 1-OHP levels as exposure biomarkers of BaP in street janitors.

4. To explore the association between BaP concentration and the emergence of urinary 1-OHP biomarker in the urine samples collected from street janitors.

1.8. Contribution to Existing Knowledge

Although several studies investigating the relationship between traffic volume and level of BaP have been done in different parts of the world (Kim et al.; 2014a; Liu et al., 2007; Wu et al., 2010; Jeng et al.; 2018b; Hemminki et al., 1994; Merlo et al., 1998; Černá et al., 1997; Ruchirawat et al., 2002; Burgaz et al., 2002; Hansen et al., 2004; Chuang, 2007; Petchpoung et al., 2011; Huang et al., 2012; Kamal et al., 2016), little research has been conducted in the Middle East (Alghamdi et al., 2015e), and particularly in Saudi Arabia (Magram, 2009). This is regardless of the religious importance of Saudi Arabia's city of Madinah, which is home to the Prophet Mohammed's Mosque. Because of this, the city is visited by a huge number of people every year, resulting in an unsually high traffic volume compared to other cities in Saudi Arabia. It is important to understand how high traffic volume contributes to both organic and inorganic pollution, and these concentrations of pollutants vary with meteorological factors. This study contributes to the existing body of knowledge by examining how traffic concentations (especially during holy months) contributes to exposure to PAHs among the residents of Madinah. Additionally, this study differs from other studies because it is based on the contribution of important religious events, such as Ramadan, in increasing the concentration of air pollutants. This has not been done elsewhere, to the best knowledge of the author.

1.9. Contribution of Acknowledged Persons

This work is an entirely orginal study by the author, including the research topic identification (driven by the knowledge gap in the existing literature), hypothesis formulation, and development of the methodology (guided by previous research, proven techniques, the availability of data, and analysis methods and equipment). Even though this work was completed entirely by the author (Khalid Nael Kordi), certain persons and organisations have played a vital role in the success of this research. My supervisors, Dr Richard McNally and Dr Anil Namdeo, guided me from the research topic identification to the completion of the work. They guided me throughout every aspect of the study.

The author obtained official approvals for collecting data from three entities: Madinah Regional Municipality, General Authority of Meteorological and Environmental Protection, and Healthy Cities Programme in Madinah. He supervised fixing and installing the high volume air samplers in the five selected sites of the study and trained the participants on using personal air samplers and gave them the instructions for its operation. He also supervised the laboratory technicians while calibrating equipments and ensuring the validity of the results. The author also made preliminary experiments for two weeks before starting the actual practical research phase (the period of collecting data) for the daily sampling operations of both urine samples and air samples from personal and high volume air samplers and for calibration of equipment and calculation of the results to ensure the commitment of participants to the instructions and the validity of equipments to be able to resolve any problem before the experiment commences. The daily withdrawing of samples and its delivery to the laboratory was supervised by the author throughout the study period. He collected data from different data points of the study (BaP and 1-OHP data from the laboratory, meterological data such as temperature, humidity, wind speed and direction from the General Authority for Meteorology and Environmental Protection and number of vehicles as well as CO concentration from the Madinah Municipality), tabulated all data and carried out the statistical analysis using SPSS version 18 and Minitab version 18, then plotted suitable graphical plots such as calendar plots using 'Openair' package version 2.6-1 with R-software version 1.1.463, time series plots using Microsoft Excel 2016 and box plots using Minitab 18.

Therefore, Madinah Regional Municipality supported this work by enabling the author to use its laboratory, provided the environmental data on air pollution, and facilitated access to the participants of the study. The contribution of the General Authority of Meteorology and Environmental Protection in Madinah to the study was also immense, as they provided the daily weather data.

1.10. Thesis Structure

Chapter 1 covers the background information, and identifies the problem being investigated. Research aim, objectives, hypotheses and study contributions are also given in chapter1.

Chapter 2 covers the scientific background and literature review.

Chapter 3 is the methodology chapter. This chapter outlines and explores the methods, procedures and techniques used to achieve the research objectives.

Chapter 4 covers results associated with BaP exposure and discussion of the results.

Chapter 5 covers the results of urinary 1-OHP concentration and discussion of the results.

Chapter 6 is the conclusion chapter. This chapter summarises the whole thesis, outlines the proposed interventions, and gives recommendations for future studies.

CHAPTER TWO

Scientific Background

Chapter 2: Scientific Background

2.1 Introduction

This chapter gives the scientific background of the research by discussing the relevant scientific aspects associated with air pollution, especially that associated with PAHs. The chapter starts by discussing the extent to which air pollution is a problem to the world, after which it then discusses how the transportation industry contributes to air pollution. It then discusses the scientific background of Polycyclic Aromatic Hydrocarbons (PAHs) in relation to their sources, types, exposure and its detection (1-OHP concentration in urine samples), the effect on human health, and factors affecting their ambient concentration. The chapter also discusses both occupational and non-occupational exposure to PAHs, how traffic volume affects the concentration of ambient PAHs, and exposure to PAHs in Saudi Arabia. Finally, the literature review and study gaps were discussed.

2.2 Air Pollution Problem

Atmospheric pollution is a worldwide problem that many nations in the world, including Saudi Arabia, are facing. It is, however, important to note that atmospheric pollution may be a global-scale problem extending from local to regional to global dimensions. In turn, this depends on the type of pollutant and its sources, as well as how it is dispersed and transported, and exposure to it (Alghamdi *et al.*, 2015a). Atmospheric pollution is thus an important aspect of public health in industrial and urban set-ups (Cariolet *et al.*, 2018). With their increasing economic power, people tend to be able to afford to purchase vehicles (new and used) (Siudek and Frankowski, 2018). Since the initial cost of electric vehicles is high compared to non-electric ones, and there is a lack of supporting infrastructure such as charging stations, people tend to acquire petrol and diesel powered vehicles rather than electric and hybrid ones (Guo *et al.*, 2020) . As a result, the general population is often exposed to pollutants associated with atmospheric pollution from vehicles.

There are a number of health-related consequences associated with continuous exposure to such pollutants, and these complications are often enhanced by microclimate conditions and synergy (Patel *et al.*, 2010a). Such conditions include cancer (Kim *et al.*, 2018), inflammation (Grunig *et al.*, 2014), respiratory complications (breathing problems) (Dasom *et al.*, 2018), and gene mutations (Kurt *et al*., 2016). Interaction with particulate matter with diameter < 10 mm (PM10) was significant for cardiovascular causes of death for all ages. The increase in the total daily number of deaths was greater in the high ozone than in low ozone days per 1 °C increase in apparent temperature which means consistent synergy could be identified in hot season and interactive effects between hot temperature and the levels of ozone and PM10 was found (Analitis *et al.*, 2018).

2.3 Contribution of Transportation to the Emissions from Air Pollutants

The rapid increase in population, urbanisation, industrialisation, insufficient public transport and increase in traffic density have signicantly contributed to uncontrolled levels of emissions in the atmosphere. As mentioned, one of the major contributors to air pollution is traffic. Figure 2-1 below shows the contribution to air pollution made by road transport, compared to other sources of pollution such as energy production, industrialisation, domestic sources, and other forms of transport in 2011 in the United States and the world. The comparison of key pollutants covers carbon monoxide (CO), benzene and hydrocarbons (HCs), particulates (soot particles and fine dust), and carbon monoxide (CO) (Katoshevski *et al.*, 2011).

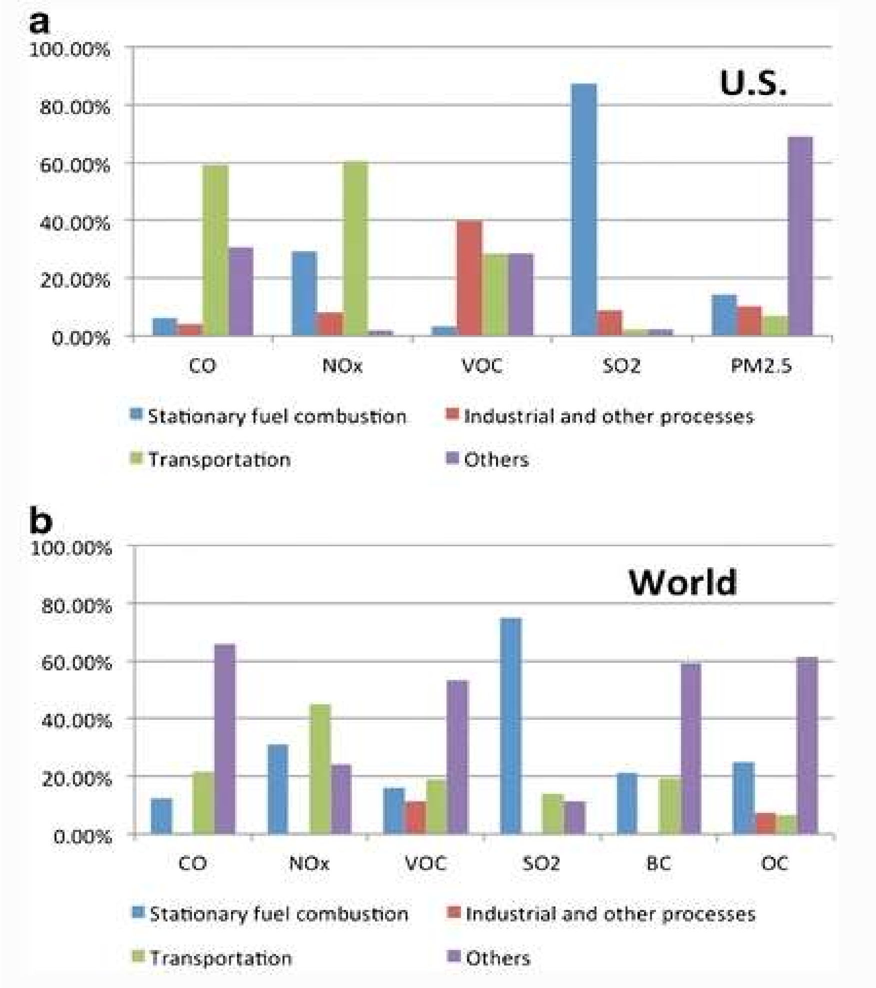


Figure 2-1: Road transport's contribution to air pollution when compared to other sources in 2010 in the United States (a) and the world (b) (Wuebbles and Sanyal, 2015).

From the above Figure 2-1, it is clear that road transport is a major contributor to air pollution. As a result, Lim *et al.* (2005a), Patel *et al.* (2010a), Katoshevski *et al.* (2011), and Liao *et al.* (2011) stated that road transport is one the most critical sources of anthropogenic emissions in urban areas. Other ways in which people can be exposed to PAHs are industrial settings, especially those who work in steel plants where boilers are fired using coal, fuel oil or other fossil fuels, and road construction, especially during alphalt production and laying (particularly when asphalt is heated before being spread on the road surface) (Cetin et al., 2018). Other sources of anthropogenic PAHs are: coke production, liquefying plants, aluminum production, coal gasification, carbon black, petroleum refineries, and catalytic cracking towers (Cetin et al., 2018).

Exposure to pollutants emitted during these activities is often associated with a number of health conditions, such as lung cancer, cardiovascular disease, and respiratory disturbances (such as asthma) (Patel et al., 2010a).

2.4 Polycyclic Aromatic Hydrocarbons (PAHs)

PAHs form a group of chemicals with two or more aromatic rings of benzene fused together (Katoshevski *et al.*, 2011). According to Katoshevski *et al.* (2011), more than 500 PAHs have been found in air. The lighter ones are often found in a gaseous state, while the heavier (the ones with more than five rings), such as benzo[a]pyrene, are absorbed into fine particulate matter (Katoshevski *et al.*, 2011). The major urban sources of PAHs are the burning of coal and vehicle transmission according to Katoshevski *et al.* (2011). The availability of PAHs in the ambient air has caused concern due to the risk of continuous exposure to large populations in industrial districts, and suburban and urban areas (Ravindra *et al.*, 2008). The PAHs that have been identified as the potential sources of carcinogens, as stated by Srogi (2007) and Kim *et al.* (2014a), are Dibenz[ah]Anthracene, Benzo[a]Anthracene and Benzo[a]Pyrene.

2.4.1 PAH Sources

According to Kim *et al.* (1999), Hu *et al.* (2007b) and Alkurdi *et al.* (2013), PAHs are mainly generated from incomplete combustion of fossil fuels. These contaminants are often ubiquitous and persistent, and one of their main sources in urban areas is vehicle emissions (Hameed, 2015; Feng et al. 2014). They can also be generated naturally, or through anthropogenic processes. The natural sources of these types of emissions, as explained by Hassan and Khoder (2012), are forest fires and volcanos, whereas the anthropogenic PAHs sources are pyrolytic processes occuring mainly as a result of incomplete combustion of organic components, power generation, residential heating, and incineration (Mastral *et al.*, 2003; Ding *et al.*, 2005; Feng *et al.*, 2014).

2.4.2 Properties and the Environmental Fate of PAHs

Bidleman (1988) explained that the concentration and other properties of PAHs in the air depend on factors such as weather conditions, strength of the source, the exchange between the particulate and gas phases, and the physical removal of the PAHs due to wet and dry depositions. Bidleman (1988) and Subramanyam *et al.* (1994) have stated that, depending on the ambient air temperature and the amount of the particulate matter suspended in air, PAHs (which are generally semi-volatile organic compounds) can exist in both particulate and gas phases in the atmosphere. Generally, low molecular weight PAHs are highly concentrated in the gas vapour phase (Zheng *et al.*, 2000), while high molecular weight PAHs tend to be associated with particulate matter (Zheng *et al.*, 2000). According to Sin *et al.* (2003), the lighter PAHs such as Phenanthrene, Anthracene and Fluorene entirely exist in the gaseous phase (more than 98% of them). The heavier PAHs such as the 5–6 ring PAHs like Benzo(a)Pyrene to Indeno(1,2,3-cd)Pyrene are usually associated with the particulate phase (where more than 75% of them exist), but the semi-volatile PAHs (four-ring) often exist in both particulate and gaseous phase various quantities. These proportions are mainly controlled by the ambient air temperature (Park *et al.*, 2002; Sin *et al.*, 2003). Because of this, most of the semi-volatile low molecular weight PAHs are found in the vapour phase (Bae *et al.*, 2002).

2.4.3 Potential PAHs

According to the US EPA, top pollutants there include many PAHs, but only 16 which have negative impacts on human health, since they can cause cancer, respiratory health complications, cardiovascular diseases, gene mutation and inflammation. These 16 PAHs are widespread and whilst there are some that have been confirmed to be carcinogens, others are suspected to be carcinogens (Cetin *et al.*, 2018). Table 2-1 below shows these 16 PAHs, their molecular weight, chemical formula and abbreviations (ATSDR, 1995). Additionally, the chemical structures of these PAHs are shown in Figure 2-2 below.

Table 2-1: Chemical identity of the most potent PAHs (ATSDR, 1995).

Chemical Identity	**Abbreviation**	**Molecular Weight**	**Chemical Formula**	**Number of Rings**
PAHs				
Acenaphthylene	ACY	152.2	$C_{12}H_{18}$	Three
Acenaphthene	ACE	154.21	$C_{12}H_{10}$	Three
Anthracene	ANT	178.2	$C_{14}H_{10}$	Three
Benzo(a)anthracene	BAA	228.3	$C_{18}H_{12}$	Four
Benzo(b)Fluoranthene	BBF	252.3	$C_{20}H_{12}$	Five
Benzo(a)Pyrene	BAP	252.3	$C_{20}H_{12}$	Five
Benzo(ghi)perylene	BGP	276.34	$C_{22}H_{12}$	Six
2-Bromonaphthalene	2-BNAP	208	$Br\text{-}C_{10}H_7$	Two
Chrysene	CRY	228.3	$C_{18}H_{12}$	Four
Dibenzo(a,h)anthracene	DBA	278.35	$C_{22}H_{14}$	Five
Fluoranthene	FLT	202.26	$C_{16}H_{10}$	Four
Fluorene	FLU	166.2	$C_{13}H_{10}$	Three
Indeno (1,2,3-cd) pyrene	IND	276.3	$C_{22}H_{12}$	Six
Naphthalene	NAP	128	$C_{10}H_8$	Two
Phenanthrene	PHE	178.2	$C_{14}H_{10}$	Three
Pyrene	PYR	202.26	$C_{16}H_{10}$	Four

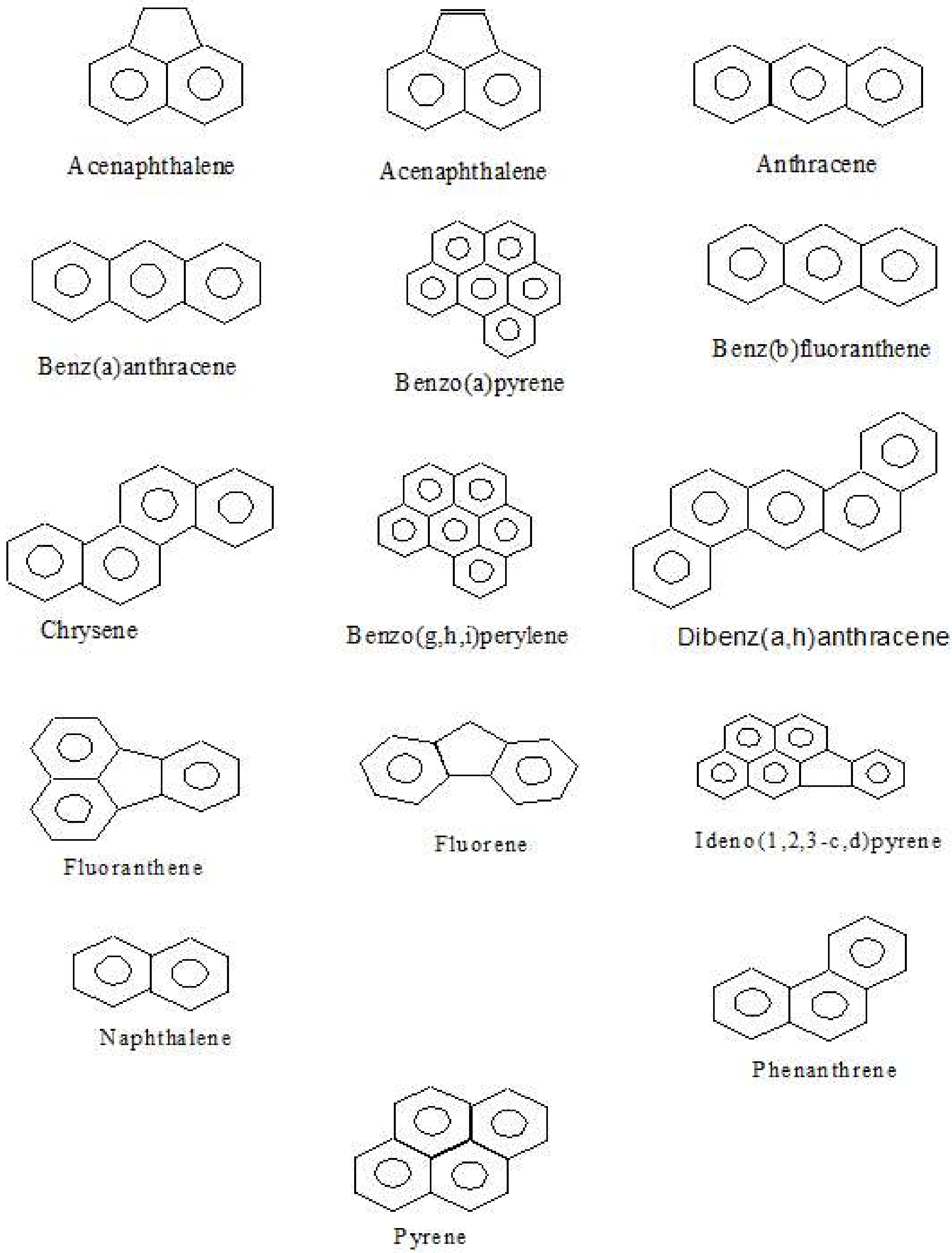


Figure 2-2: Ring structure of the various types of PAHs (*source:* ATSDR (1995)**.**

2.4.4 Benzo[a]pyrene (BaP)

BaP is considered one of the most critical compounds belonging to PAHs. It is associated with incomplete burning of organic materials at temperatures ranging from 300 ºC to 600 °C (Zheng *et al.*, 2000). It is often found in coal, tar, many types of food such as grilled meats, and in tobacco smoke (Zheng *et al.*, 2000). It is formed when a benzene ring is fused to pyrene. Since its diol epoxide metabolites react with and bind to DNA, it results in mutations and eventually causes cancer (Bae *et al.*, 2002). It also affects the number of white blood cells since it inhibits some of these cells from differentiating into macrophages, which is the body's first line of defence in fighting infections (Bae *et al.*, 2002). It is categorised as a group 1 carcinogen because there is sufficient evidence that it can cause cancer (Bae *et al.*, 2002).

The evidence that BaP is carcinogenic is provided by Goyal *et al.* (2010), who, while studying the Syzygium cummini extract as an anti-cancer agent, injected mice with benzo-a-pyrene to induce stomach carcinogenesis. The research showed the injection of BaP did indeed induce stomach cancer and there was also evidence that Syzygium cummini extract might be used as an anti-cancer or anti-tumour agent.

Chen *et al.* (2013) conducted a study to associate BaP with DNA mismatch repair in human breast cancer cells. The study found that exposure to BaP inhibits DNA mismatch repair activity in ZR75-1 cells. Chen *et al.* (2013) therefore concluded that DNA mismatch repair activity induced by BaP enhances mutagenesis progression. Ronco *et al.* (2011) carried out a study to associate dietary Benzo[a]pyrene and human breast cancer in Uruguay. The study found that BaP exposure is significantly associated with breast cancer cases, and therefore its exposure is asssociated with high risk of developing breast cancer.

2.4.5 Regulation

Table 2-2 shows the limit values of BaP in the air from several regulations. For instance, the WHO has set the reference level for BaP concentrations as 0.12 ng m^{-3}, which was estimated by assuming a WHO unit risk for lung cancer for PAHs mixtures (WHO, 2003). According to the European Environment Agency the target value for mean annual BaP concentrations for minimizing human health risk is set at 1 ng m^{-3} (EEA, 2016). There is a discrepancy between the reference level of BaP among countries; for example, in China it is 1 ng m^{-3}, which is similar to European limits, while in the United States there are three category references, as shown in Table 2-2. In addition, according to the Ministry for Environment (MFE), New Zealand has a reference level of BaP 0.3 ng m^{-3} (MFE of New Zealand, 2003). It is noteworthy that there are no standard standards belonging to Saudi Arabia, as it relies directly on the European standard limits (Bazzaz *et al.*, 2011).

Table 2-2: Limit values of BaP in Air

Organization or Journal	Location	References	BaP	Environmental standards
European Environment Agency	EU	EEA (2016)	1 ng m^{-3}	Annual EU target value (EU 2004)
WHO	-	WHO (2003)	0.12 ng m^{-3}	Annual WHO reference level (WHO 2013)
MFE of New Zealand (2003)	NZ	(MFE of New Zealand, 2003)	0.3 ng m^{-3}	Annual environmental standard
MEP of China (2012)	China	China (2012)	1 ng m^{-3}	Annual ambient air quality standard
The occupational safety and health administration (OSHA)	USA	USEPA (2013)	0.2 mg m^{-3}	An 8-h time-weighted permissible exposure level
The National Institute for Occupational Safety and Health (NIOSH)	USA	USEPA (2013)	0.1 mg m^{-3}	8-hr time weighted permissible exposure level for coal tar pitch volatile agents
American Conference of Governmental Industrial Hygienists	USA	USEPA (2013)	0.2 mg m^{-3}	Threshold limit value for a normal 8-h workday and a 40-h workweek, to which nearly all workers may be repeatedly exposed

2.4.6 Carcinogenic Classification of PAHs

Because of the carcinogenic and persistence properties of PAHs, they are categorized according to their carcinogenic properties. Table 2-3 below shows the carcinogenic classification of selected PAHs.

Table 2-3: Carcinogenic classifications of selected PAHs by specific agencies

Order	Agency	PAH compound(s)	Carcinogenic classification	Reference
1	Agency for Toxic Substances and Disease Registry (ATSDR)	• Dibenz(a,h)anthracene • Indeno(1,2,3- c,d)pyrene • Benz(a)anthracene • Benzo(b)fluoranthene • Benzo(a)pyrene	Known animal carcinogens	(ATSDR, 1995)
		• Benzo(a)pyrene • Benz(a)anthracene and	Probably carcinogenic to humans	
2	U.S. Environmental Protection Agency (EPA)	• Benz(a)anthracene • Benzo(a)pyrene • Benzo(b)fluoranthene • Benzo(k)fluoranthene • Chrysene, • Dibenz(a,h)anthracene, and • Indeno(1,2,3-c,d)pyrene	Probable human carcinogens	(USEPA, 2008)
		• Benzo(g,h,i)perylene • Fluoranthene • Acenaphthylene • Anthracene	Not classifiable as to human carcinogenicity	
3	International Agency for Research on Cancer (IARC)	• Benzo(k)fluoranthene • Ideno(1,2,3-c,d)pyrene • Benzo(a)fluoranthene	Possibly carcinogenic to humans	(IARC, 2010)
		• Benzo(g,h,i)perylene • Benzo(e)pyrene • Fluoranthene • Fluorene • Phenanthrene • Pyrene • Anthracene • Chrysene	Not classifiable as to their carcinogenicity to humans	

2.5 Biomonitoring Programmes

According to the ACC (American Chemistry Council) (2011) biomonitoring is the determination or measurement of the burden of toxic elements, chemicals, compounds and their associated metabolites in the body by the use of biological substances. Even though exposure to PAHs can be determined with less effort by measuring airborne PAHs, biological monitoring takes care of all the emission sources, as well as the routes through which these contaminants are adsorbed into the body. It also takes care of the efficiency of protective equipment and genetic and non-genetic factors. This is one of the reasons why the American Conference of Governmental Industrial Hygienists endorsed monitoring of the levels of 1-OHP in urine samples of affected population as a biological exposure index for examining PAH exposure (Heikkilä *et al.*, 1995; ACGIH, 2005a)

2.5.1 Urinary 1-hydroxypyrene as a biomarker for PAHs

In order to test the extent of human exposure to PAHs, a number of biomarkers can be used. These biomarkers include urinary metabolites, PAH-Protein adducts, the PAHs gene-toxic end points in the lymphocytes, and the PAH-DNA adducts. Of these methods, Ifegwu *et al.* (2012) state that the most extensively used technique as an indicator of the exposure to PAHs is excretory 1-hydroxypyene (1-OHP), which is a metabolite of pyrene (urinary metabolite). The questions are: what is a biomarker, and why is 1-OHP extensively used as a biomarker of PAHs exposure?

2.5.2 What is a Biomarker?

According to Ifegwu *et al.* (2012), a biomarker is generally a physiological, biochemical, behavioural or other form of change (or alteration) which occurs within an organism (in this case human being) that can be detected and recognised as being associated with a possible or established health problem or condition, and disease or impairment. Strimbu and Tavel (2010) define a biomarker as medical sign that is an indication of a medical state (condition) being observed from outside a patient, and which can be accurately measured and reproduced. However, this definition by Strimbu and Tavel (2010) tends to be deficient, as it states that a biomarker is a medical sign that can be associated with a medical condition being observed from outside a patient. This is because the medical condition does not need to observed in this way. For instance, exposure to toxic substances may not have an immediate effect on an individual but can be detected long before medical problems associated with the exposure start to manifest in the patient. Biomarkers can also be defined as a property that can be objectively measured, assessed and evaluated as a sign of pathogenic processes, normal biological processes, or pharmocological responses of an individual or animal to a therapeutic intervention (Strimbu and Tavel, 2010). The WHO has defined biomarkers as “any substance, structure, or process that can be measured in the body or its products and influence or predict the incidence of outcome or disease” (Strimbu and Tavel, 2010).

2.5.3 Why 1-OHP is Extensively Used as a Biomarker for PAHs Exposure

As stated earlier, one of the most extensively used biomarkers for detection of PAHs exposure is 1-OHP. One of the major reasons as to why 1-OHP is one of the most commonly used biomarkers for the detection of PAHs is that it is a major product of pyrene metabolism, which is abundant in PAH mixtures (Lu *et al.*, 2016) and, in fact, represents 90% of pyrene metabolites. The metabolism of pyrene to form 1-OHP is shown in Figure 2-3 below.

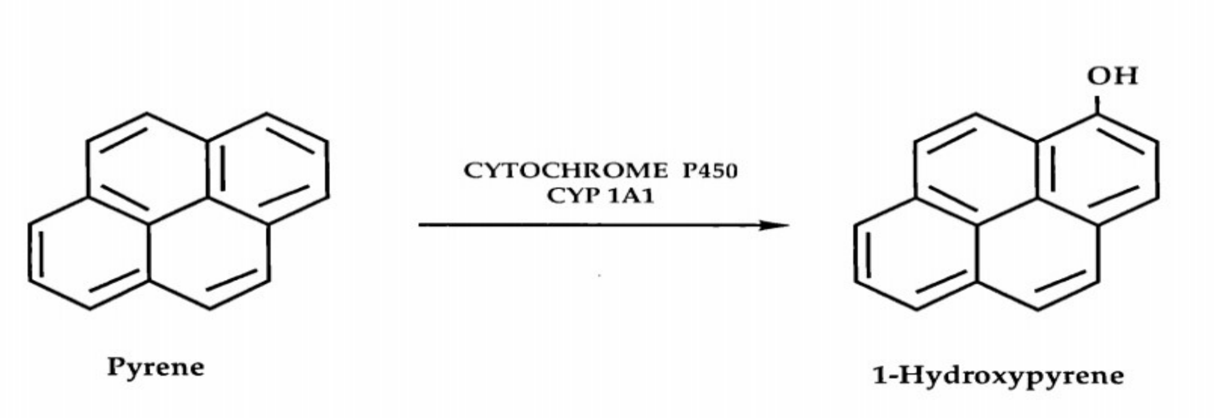


Figure 2-3: Metabolism of Pyrene to 1-Hydroxypyrene (*source:* ATSDR (1995))

The second reason is that it has a relatively short half-life compared to other biomarkers, which makes it appropriate for the detection of short-time PAHs exposures. According to Kim *et al.* (2014a), 1-OHP has a half-life ranging between 18–20 hours, which means it can manifest itself in urine 24 hours after exposure. Other methods that can be used as biomarkers such as PAH adducts have an extended half-life (Castano-Vinyals et al., 2004). For instance, according to Castano-Vinyals *et al.* (2004), the half-life of the DNA adducts in the lymphocytes is usually in the order of months. According to Castano-Vinyals *et al.* (2004), the half-life of protein adducts often depends on the protein type where the PAH chemical is attached. For instance, in the case of haemoglobin adducts, the half-life is approximately 120 days, and in the case of albumin adducts, the half-life is approximately 20 days (Castano-Vinyals *et al.* (2004).

Another reason as to why 1-OHP is one of the most commonly used biomarkers for detecting PAHs is that, compared to other PAHs, the content of pyrene (which is its major by-product) is relatively constant in both work place environment air samples and dermal samples (Alshaarawy *et al.*, 2014). Another reason, according to Jeng *et al.* (2018b), is that 1-OHP can be detected in urine with relatively high sensitivity. Pyrene is often absorbed into the body through the skin, lungs and gastrointestinal tract (Recio-Vega *et al.*, 2018). After absorption, it is metabolised to 1-hydroxypyene (1-OHP), which is then excreted in urine (Alshaarawy *et al.*, 2014).

2.5.4 The Relationship between 1-OHP Levels and Exposure to PAHs

Studies have been carried out to determine the relationship between the level of exposure to 16 PAHs (those PAHs with the most significant negative impacts on human health) and the levels of 1-OHP detected. For instance, Jeng *et al.* (2018b), carried out a study to determine the statistical relationship between 1-OHP levels and exposure to the 16 PAHs. The study was carried out on coke-oven workers, and 100 participants were involved. The urine samples from the participants were collected in sterilized 50 mL polypropylene cups and stored at -80 ºC before being analyzed for 1-OHP using an HPLC with a fluorescent detector. The results of the study indicated there was a positive and statistically significant relationship between 1-OHP levels and exposure to all the 16 PAHs. These results are shown in Table 2-4 below.

Table 2-4: Correlation between exposure levels of the 16 PAHs and 1OHP levels (Jeng *et al.*, 2018a)

PAH	Correlation	
	Pearson's Correlation Coefficient	p-value
Pyrene	0.596	<0.0001
Phenanthrene	0.67	<0.0001
Naphthalene	0.479	0.0013
Indeno(1,2,3-cd)pyrene	0.567	<0.0001
Fluorene	0.591	<0.0001
Fluoranthene	0.629	<0.0001
Dibenz(a,h)anthracene	0.579	<0.0001
Chrysene	0.578	<0.0001
Benzo(k)fluoranthene	0.578	<0.0001
Benzo(g,h,i)perylene	0.577	<0.0001
Benzo(b)fluoranthene	0.598	<0.0001
Benzo(a)pyrene	0.602	<0.0001
Benzo(a)anthracene	0.595	<0.0001
Anthracene	0.598	<0.0001
Acenaphthylene	0.601	<0.0001
Acenaphthene	0.568	<0.0001
Total PAHs	**0.623**	**<0.0001**

From Table 2-4, it can be observed that there are positive Pearson's correlation coefficients associated with the relationship between the level of 1-OHP and exposure levels of all the PAHs. The overall relationship, which is statistically significant, has a coefficient of 0.623 and p value of 0.0001. These results undoubtedly suggest that the levels of 1-OHP detected in urine can be used as a bio-signature for detecting human exposure to PAHs, and the risk of health complications as a result of the exposure.

Kim *et al.* (2014a) also carried out a study to determine the relationship between levels of urinary 1-OHP and occupational exposure to airborne PAHs. The study found that levels of urinary

1-OHP are positively correlated with occupational exposure to airborne PAHs; therefore, high concentrations of PAHs in urine can be used as a reliable bio-signature (biomarker) for occupational PAH exposure.

Liu *et al.* (2007) also carried out a study to determine this relationship. The researchers used regression analysis to investigate if there was indeed a relationship between levels of urinary 1-OHP and occupational exposure to PAHs. They studied about 75 coke-oven workers in companies in Taiwan. The regression analysis showed that there is a positive relationship between levels of 1-OHP and concentration of PAHs in the air.

Wu *et al.* (2010) also carried a study to investigate the relationship between exposure of workers to BSF (benzene soluble fraction), which is a PAH, and 1-OHP level on 80 Taiwan coke-oven employees. Wu *et al.* (2010) carried out a Pearson correlation analysis to determine this relationship, the results of which found a positive relationship between post-shift levels of urinary 1-OHP and BSF in the air. The correlation coefficient was found to be 0.74.

2.5.4.1 Summary of the study details

The details of the above studies are summarised in Table 2-5a.

Table 2-5a: Summary of studies regarding the relationship between 1-OHP levels and exposure to PAHs

Authors	Country/Place	Participants	Exposure methods
Jeng *et al.* (2018b)	Southern Taiwan	Coke-oven workers	Inhalation and dermal exposure
Kim *et al.* (2014a)	New York, United States	Cigarette smokers	Inhalation and ingestion
Liu *et al.* (2007)	Taiwan	Coke-oven workers	Inhalation and dermal exposure
Wu et al. (2010	Taiwan	Coke-oven workers	Inhalation and dermal exposure

2.5.5 Measurement of the Relationship between 1-OHP and PAH Exposure Associated with Urban Pollution

While in the industrial sector, the concentration of PAHs is measured in terms of the concentration of PAHs in the work environment and correlated with the level of 1-OHP in the urine of workers involved in the study. In the environmental exposure associated with exposure of PAHs as result of urban pollution, the amount of 1-OHP excreted together with urine is correlated with the total quantity of particulate matter in air and "breathing fraction concentration particulate matter in the air" (Jeng *et al.*, 2018b).

2.5.6 Limitations of Using 1-OHP as PAHs Exposure Biomarker

Even though 1-OHP is an excellent biomarker for the detection of exposure to PAHs, it is also associated with a number of limitations. According to Alshaarawy *et al.* (2014), the use of urinary 1-OHP to detect PAHs only detects recent PAH exposure, and cannot be used to determine the difference between past PAH and current PAH exposures. Secondly, the assessment of volatile PAH exposures using level 1-OHP may be ineffective, as it is only valid when pryene is present (Jeng & Pan, 2015). However, given that pyrene is predominant in most PAHs and in the particulate phase of air, 1-OHP is an excellent biomarker for semi-volatile PAHs as well as pyrene (Alshaarawy *et al.*, 2014).

2.6 Comparison between Occupational and Non-Occupational PAH Exposures

According to Alshaarawy *et al.* (2014) and Ifegwu *et al.* (2012), the use of 1-OHP in detecting the exposure to PAHs can also be applied in environments with low levels of PAHs. This means that in addition to the fact 1-OHP can be used to detect PAH exposure in occupational environments, it can also be used to detect low levels of PAHs, which are often associated with non-occupational exposure to PAHs (Alshaarawy *et al.*, 2014). It is, however, important to note that there was no evidence of a relationship between urinary 1-OHP levels and PAH levels in non-occupational environments as in occupational environments. As stated by Ifegwu *et al.* (2012), one of the reasons for this is that, in occupational settings, the levels of PAHs are normally elevated, and therefore people are likely to be exposed to more PAH levels in occupational settings than in non-occupational settings.

2.7 Health Problems Associated with PAH Exposure

Human exposure to PAHs is a great concern due to the carcinogenic and mutagenic properties (Tang, 2008; Haritash and Kaushik, 2009; Dvorská *et al.*, 2012). These compounds can cause both chronic and acute health complications depending on the route of exposure, period of exposure, PAH concentration, and toxicity. According to Kim *et al.* (2014a), long term exposure to PAHs such as BaP and pyrene is often associated with cancer, and, as stated by Unwin *et al.* (2006), the penetration of PAHs into the body via the lungs, cutaneous routes of absorption, and ingestion has mutagenic, endocrine disrupting, and carcinogenic effects on people and wild life. Other routes of exposure are dermal, inhalation, and ingestion (Ravindra *et al.*, 2008). Additionally, exposure to PAHs by the general population through breathing smoke in open fireplaces, indoor air saturated with cigarette smoke, and/ or eating foods containing PAHs are considered direct exposure to risks associated with PAHs (ACGIH, 2005a; CRCE, 2008). People may also be exposed to PAHs at their work places (occupational exposure), such as mechanics, drivers and street vendors, who may breathe in exhaust fumes (See *et al.*, 2006). Also, those involved in mining, metal working and oil refining are exposed to PAHs at work (See *et al.*, 2006).

The toxicity of the PAHs, according to Lodovici and Bigagli (2011), is often associated with their biotransformation into reactive metabolites, which results in the production of a reactive oxygen species capable of inducing protein and lipid (fat) oxidation, the depletion of endogenous antioxidants in an organism associated with diabetes mellitus (Alshaarawy *et al.*, 2014; Kim *et al.*, 2014a), cardiovascular complications (Feng *et al.*, 2014; Hu *et al.*, 2015a), and metabolic syndromes (Brocato *et al.*, 2014; Hu *et al.*, 2015a).

The carcinogenic effects depend on the exposure to PAHs, such that the longer the exposure period, the higher the chance of developing cancer. Therefore, in order to help fight cancer, which is currently a major health concern, the exposure to PAHs needs to be below the exposure limit levels or even zero. However, owing to the importance of fossil fuel in the current world, despite the existence of greener energy techologies, achieving zero exposure to the may not be possible now (Ravindra et al., 2008).

2.7.1 1-OHP Concentration and Risk of Cancer

Cancer arises as a result of the interaction between a person's genetic factors and environmental factors including physical, chemical, and biological carcinogens. Physical carcinogens include different types of radiation, while biological carcinogens include viral or bacterial infections. On the other hand, chemical carcinogens include heavy metals, dioxins, aflatoxins and PAHs. Among those chemical carcinogens; PAHs were ranked the ninth most threatening compounds to human health in 2001 (Šimko, 2002) and the largest class of chemical compounds known to be cancer-causing agents. The most prominent source of PAHs are petroleum-related sources as confirmed by Autrup *et al.* (1999) who reported high urinary 1-OHP level (0.25 μg mol/mol creatinine) for suburban/rural bus drivers. Other researchers have also reported higher urinary 1-OHP in taxi drivers more than professional drivers and commercial drivers (Burgaz *et al.*, 2002).

Although 1-HOP is commonly used as a biomarker for PAH exposure, it is not specific for air pollution but can also be found in food. Moreover, several researchers found smoking to be correlated with an elevated urinary 1-OHP as confirmed by Merlo *et al.* (1998) who found that the average 1-OHP for traffic officers who smoked was higher than their non-smoking counterparts. In addition, cigarette smoking has been reported to significantly increase urinary 1-OHP concentrations and has been linked to lung, head and neck cancers. Several studies have linked exposure to high PAH concentrations to cancer. In China, researchers found that the mortality rate from lung cancer was five times the Chinese national average especially among women. This was attributed to the high atmospheric PAHs arising from the use of smoky coal as fuel (Xu *et al.*, 2006). Various studies indicate the possibility of future cancer cases as a result of occupational exposure. Increased risk of lung tumours was observed among workers in aluminum smelters (Armstrong and Gibbs, 2009), pavers and roofers (Boffetta *et al.*, 2001). Moreover, an association between PAH-DNA adducts and tumours of the stomach, bladder, skin, leukemia and breast cancer were also observed (Gammon *et al.*, 2004).

Epidemiologically, adverse health problems such as" asthma, cardiovascular diseases and cancers" have multifactorial aetiological factors. These factors include ambient air pollution, smoking and occupational exposure (Samet et al., 2000; HEI, 2002; EOM et al., 2013; Gass et al., 2015). Occupational exposure and generally low concentrations of specific environmental pollutants may be associated with elevated risk of cancer and other health problems. However separating-out these etiological factors and assessing their quantitative impact on health is still critical, because a major reduction in disease will only result if all/ or most of the important etiological factors are diminished/ or removed (Carnow, 2018).

Generally environmental pollution is considered an important factor in the development of cancer and cardivascular diseases. The National Academy of Sciences in its monograph, Particulate, Polycyclic Organic Matter, suggested that polycyclic organic compounds in the form of air pollutants were a significant factor in the etiology of lung cancer (Pollutants, 2015). It was estimated that the effect of air pollution on pulmonary cancer death rate of a 5% increase per unit increase in urban air pollution, as indexed by benzo[a]pyrene (BaP), one BaP unit equals lμg of BaP per 1000 m^3 of air (Carnow and Meier, 2007). Toxicological evidence indicates that benzo[a]pyrene (BaP) is one of the most abundant PAHs found in the environment and is present in almost all mixtures of PAHs in relatively high concentrations (2%–10%) (Yang *et al.*, 2000) Humans are commonly exposed to PAH mixtures through the inhalation of polluted air and cigarette smoke (Fan *et al.*, 2012b). Polycyclic aromatic hydrocarbons (PAHs) are one class of chemical compounds generated during cigarette smoking (Hoffmann, 1997).

Epidemiological studieshave found higher levels of 1-OHP in the urine of smokers than non-smokers (Huang *et al.*, 2012). Cigarette smoking is by far the strongest, in fact, the overwhelming factor causing lung cancer. It is estimated that smoking contributes ~ 80–90% of lung cancer deaths, almost doubling of lung cance mortality in large cities compared to rural areas, in USA (29.4 versus 14.6), and in examining cigarette smoking among these population, ~ 48.5% of adults in the urban areas smoked cigarettes compared to 42.5% in rural areas (NIOSH, 2019). Other urban-rural studies give no cigarette data, but show significant differences, almost a doubling, shifted towards the urban population. One study showed a difference in ratios between London and Wales of 1.37 to 0.79 for males, and 1.32 to 0.69 for females (Curwen *et al.*, 2005; Levin *et al.*, 2007) concluded thatthere was a gradient between rural lung cancer death rates (15.2%) to metropolitan urban areas lung cancer death rates, away from central city, (20.8%), to city-urban lung cancer death rates (29.2%) a doubling of percents of death rates from rural to urban with an intermediate in the urban, noncity area. Smoking is established as risk factor for lung cancers, this risk was at least 20 % higher in people residing in urban than rural areas (Secretan et al., 2009). Moreover it was appeared that people who leaving in the United Kingdom were shown to have a 35% increase in lung cancer, if they left prior to the age of 30, and a 75% increase in lung cancer if they left after the age of 30, suggesting the influence of environmental-pollution exposure (Carnow, 2018).

In spite of many studies havefound variations between cancer incidence in urban and rural areas,, higher incidence shifted towards the urban areas (Wilkinson and Cameron, 2004; Riaz *et al*., 2011; EOM *et al*., 2013), this is not a universal finding, and the incidence patterns differ by country, time period, gender and type of carcinogenic air factors (Yang and Hsieh, 2001; Obertova *et al.*, 2012). This confirming that exposure to cancer risk factor (e.g. tobaco smoke) may be varied across socioeconomic groups (Kogevinas *et al*., 2002).

The difference in socioeconomic composition of urban and rural areas (e.g. lifestyle, risk factors, income, occupation, education) reflects the variation in cancer incidences between urban and rural areas. The risks of breast, cervical, kidney and brain cancer were significantly higher in females in urban areas however prostate cancer risk was higher in rural areas (Sharp *et al*., 2014). Smoking is established a risk factor for cancers (head and neck, esophagus, stomach, lung, bladder and cervix) in people residing in urban compared to rural areas (Secretan *et al*., 2009) (IARC, 2010).

PAHs enter the environment by several pathways, and are present in unburned petroleum (petrogenic PAHs) and can be released directly to the environment both by human activities and natural processes. Combustion particles from vehicles were associated with increased mortality whereas fine particulates derived from crustal sources were not associated with increased risk, in Six U.S. Cities (Laden *et al.* (2006). Open burning of biomass and disposal of household/ or agricultural wastes are concerned to air quality and emission of toxic pollutants, in the rural areas (Isley et al., 2016). Land use and air pollution are closely linked (WHO, 2016). According to the World Health Organization (WHO) air pollution is the preponderant environmental risk factor, being responsible for about one in every nine deaths globally (WHO, 2016).

Various kinds of pollution are produced depending on land-use and human activity, contributing various air quality and health effects (Bank, 2012). A number of factors affects PM toxicity, including size, shape, structure, surface reactivity, bio-persistence and presence of soluble components (Cassee *et al.*, 2013). These factors are highly variable depending on with activity, time, and location, and (Watson *et al.*, 1988a). Combustion engines, in particular diesel engines are major contributors to $PM_{2.5}$ in urban environments. Diesel exhaust particles have the ability to form reactive oxygen species (Hiura *et al.*, 2000). Organic and metal associated particlate matter can induce proinflammatory effects in the lung, due to their ability to cause oxidative stress (Nel *et al*., 2001; Saldiva *et al*., 2002). In addition PAH compoundsinduce oxidative stress indirectly, through biotransformation by cytochrome P450, expoxide hydrolase, and dihydrodiol dehydrogenase to generate redox active quinones (Penning *et al*., 1999).

PAHs are well know as carcinogenic, mutagenic, teratogenic, and genotoxic substances and they have associated with breast, and brain cancers (Ravindra *et al.*, 2008; Sánchez-Guerra *et al.*, 2012; Mielzynska-Svach *et al.*, 2013; Yamano *et al.*, 2014). Although several studies have found relationships between PAHs exposure and cancers, the causalrelationship between the level of 1-OHP and cancer incidences is limited, particularly in the developing countries. Some studies have found relationships between urinary 1-OHP and genotoxic effects on target tissues and cells (sister chromatid exchanges and frequency cells). Mielzynska-Svach *et al*. (2013) found positive correlations between concentrations of PAHs and 1-OHP levels in urine; using cytokinesis-block micronucleus cytone assay, *Salmonella* mutagenicity assay and an Ames test 1-OHP; and cancer incidences. Some of the PAH compounds are tumorigenic (Yamano *et al*., 2014), they have ability to generate DNA adduct, causing oxidative damage to the DNA as a result of the production of a reactive oxygen [8-oxo-7,8-dihydro-2-deoxyguanosine (8-oxodG)] during the metabolism process (Huang *et al*., 2012; Jeng and Pan, 2015). This damage is often caused by the reaction between guanosine located in the C-8 location in the gene and hydroxyl radicals, causing mutations through the G to T transversion (Buchet *et al*., 1995). Moreover 8-oxodG is used as a biomarker to evaluate DNA damage associated with PAHs exposure and cancer risks (Wu *et al*. (2010), and to evaluate the rate of DNA damage, and the difference between DNA repair and damage rates (Jeng and Pan, 2015).

2.7.2 1-OHP Level and Risk of Respiratory Diseases

Studies have found a relationship between PAH exposure and respiratory problems, mainly due to the fact that PAHs are often adsorbed to the reactive surfaces of the particulate matter suspended in air, which, according Prado *et al.* (2012), can result in respiratory complications. Research has been carried out to determine if the concentrations of 1-OHP in the urine can be used to evaluate the relationship between respiratory health and air pollution associated with particulate matter. For instance, a study was carried out on healthy sugarcane workers in Brazil (Mendonca) to evaluate the respiratory symptoms of these workers as a result of exposure to particulate matter. The workers who experienced elevated coughing, sneezing, overall reduction in the pulmonary function, and elevated levels of wheezing had higher spirometer readings. When the relationship between these readings and 1-OHP levels in the urine was determined, there was a negative relationship between spirometer values and 1-OHP concentration in urine. This means that the people with respiratory conditions as a result of PAH exposure have greater chances of exhibiting higher levels of 1-OHP. Therefore, according to this study there is a positive relationship between 1-OHP concentration and the risk of contracting respiratory health complications, especially when one is exposed to PAHs.

Choi *et al.* (2013) also carried out a study to determine the relationship between decreased pulmonary function and 1-OHP levels in urine amongst elderly people in a community. They found a negative relationship between pulmonary function and 1-OPH level in these people, and, as with the previous study, this means that there is a positive relationship between 1-OHP concentration and the risk of contracting respiratory health complications, especially when one is exposed to PAHs.

Even though there are studies that show the existence of a positive relationship between 1-OHP concentration and the risk of contracting respiratory health complications when an individual is exposed to the PAHs (which may either be associated with work station or accidental exposure), some studies have found no relationship between these two factors (Mielzynska-Svach *et al.*, 2013).

2.7.3 1-OHP Concentration and Urine and Risk of Cardiovascular Disease

Studies have shown that there is an association between exposure to PAHS and the risk of developing cardiovascular disease or the risk of cardiopulmonary mortality. According to Curfs *et al.* (2005), exposure to PAHs significantly increases the volume of aortic plaque, and the authors further state that exposure to PAHs also increases the volume of the inflammatory cells found in atherosclerotic plaques. Burstyn *et al.* (2005) stated that workers in work environments associated with a high concentration of PAHs have increased risk of contracting ischemic heart disease, with these types of workers also associated with cardiovascular mortality (that is, most of them die of cardiovascular-related complications). The Burstyn *et al.* (2005) study, however, did not include the exposure of individuals who live near these organisations rather than work in companies associated with high PAH emissions, and so their family members are also at risk of PAH exposure and contracting cardiovascular complications associated with the exposure.

Prado et al. (2012) explain that PAHs tend to induce inflammation and oxidative stress on cardiovascular tissues and cells. There are several ways of detecting the effect that exposure to PAHs has on a person’s cardiovascular health. One such method, according to Jeng and Pan (2015), is by using 1-OHP. For instance, Brucker *et al.* (2013) used the concentration of 1-OHP in urine as a biomarker for evaluating the relationship between the risk of cardiovascular medical complications and exposure to PAHs. They conducted this experiment on taxi drivers to determine the association of PAH exposure with cardiovascular complications, concluding that there is a positive correlation between inflammatory cytokines and the concentration of 1-OHP in urine as well as elevated levels of pro-inflammatory cytokines. The study further concluded that PAHs can cause cardisovascular diseases in people frequently exposed to benzo(a)pyrene and particulate matter, compared to those who are not. Lee *et al.* (2011) used the relationship between 24-h ambulatory electrocardiogram patterns and 1-OHP concentrations to find an association between occupational exposure to PAHs and alteration of the cardiac autonomic function in individuals who make boilers.

Clerk et al. (2012) carried out a study in the United States in which CRP (C-reactive protein) measurements were recorded during the National Health and Nutrition Examination Survey (NHANES) of 2003–2004, and evaluated the role of urinary 1-OHP in atheroclerosis progression. The results showed a positive relationship between 1-OHP levels and the CRP mesurements, but the study never included other health complications that might be associated with PAH exposure, such as cancer and inflammation.

In summary, it is however important to note that, while exposure to air pollutants such as PAHs can cause cardiovascular diseases and conditions, this does not necessarily cause cardiovascular mortality, as there are other factors that can cause cardiovascular death (Prado et al., 2012), such as heart attack, stroke, consumption of alcohol, unhealthy diet, and lack of exercise.

2.7.4 Association between 1-OHP Concentration in Urine and Inflammation

A number of studies have shown evidence linking allergic and enhanced systematic inflammation with exposure to PAHs. Jeng *et al.* (2011) reported that human exposure to PAHs can alter the production of immunoglobulins and cytokines, and showed that 1-OHP concentration levels in workers in coke ovens is related to their IgE levels. The study, however, found a weak relationship between IgG and IgA and the concentration of 1-OHP in urine of the workers.

Alshaarawy *et al.* (2013) studied the relationship between low background exposure to PAHs and inflammation to a general population using data collected from the National Health and Nutrition Examination Survey between 2001 and 2006 in the United States of America. They reported a positive relationship betweeen WBC count (white blood cell count) and CRP, and concentration of 1-OHP in the urine of the participants. Everett *et al.* (2010) studied the relationship between the elevated levels of CRP and low exposure to PAHs, amongst 999 participants using National Health and Nutrition Examination Survey data collected between 2003–2004 in the United States. The study reported a positive relationship between elevated levels of urinary 1-OHP in individuals exposed to low levels of PAHs and elevated levels of CRP (serum CRP).

Leem *et al.* (2005) carried out a cross sectional study to investigate the relationship between exposure to PAHs, and atherogenesis and inflammation in taxi drivers. This study reported increased levels of unirary 1-OHP in the taxi drivers exposed to higher levels of PAHs, compared to participants exposed to lower levels of PAHs (i.e. individuals not exposed via their occupation). Leem *et al.* (2005) also found that PAHs are associated with anti-inflammatory cytokines and pro-inflammatory during asthma attacks. That is, concentration of urinary 1-OHP is positively related to lipid peroxidation and inflammatory cytokines, such as IL-6.

2.7.5 Association between Urinary 1-OHP Concentration and Reproductive Health

Han *et al.* (2010) and Wu *et al.* (2010) reported that exposure to PAHs can be linked to male infertility as a result of a decrease in the sperm quality and interference with sperm DNA, with it also being reported that exposure to PAHs can be linked to increased incidences of miscarriage in pregnant women. According to Orjuela *et al.* (2010), when pregnant women are exposed to PAHs, the number of chromosomal aberrations is increased in their newborns. Also, when Xia *et al.* (2009) carried out a study to determine the relationship between exposure to PAHs and reduction in fertility in men. The study found that men who exhibited higher levels of 1-OHP had low sperm count and quality per ejaculum. In fact, these men's sperm count per ejaculation was lower than recommended by the WHO (Xia *et al.*, 2009).

Ji *et al.* (2010) also carried out a study to determine the effects of exposure to PAHs on sperm quality, particularly the quality of the sperm DNA. The study was carried out on male participants who had unexplained male infertility, finding that the subjects who exhibited higher concentrations of urinary 1-OHP also exhibited higher levels of DNA adducts. That is, subjects who exhibited a higher concentration of the 1-OHP in their urine also had higher levels of sperm DNA fragmentation. Therefore, according to this study, it can be concluded that there is a positive relationship between PAH–DNA adducts and the concentration of urinary 1-OHP.

Jeng *et al.* (2013a) carried out a study to investigate whether exposure to PAHs can be associated with a reduction in the quality of semen as well the DNA quality of the sperm. The study was carried out on non-smoking coke oven workers, reporting a positive relationship between exposure to the 16 PAHs and concentration of urinary 1-OHP levels amongst the participants. The study did not, however, find a significant relationship between semen quality and the concentration of urinary 1-OHP levels, nor did it find a significant relationship between the concentration of 1-

OHP in the urine of the participants and DNA fragmentation. What it did find was a significant relationship between the concentration of unrinary 1-OHP and bulky and oxidised DNA adducts. Han *et al.* (2010) also found no significant relationship between the concentration of urinary 1-OHP and semen quality, for the parameters of viability and morphology.

It has been observed that the results of the study carried out to determine the relationship between exposure to PAHs and sperm and semen quality are very inconsistent. This, according to Han et al. (2010), is due to the variations in the metabolic pathways as well as the effect of sperm repair mechanisms. Prado et al. (2012) explained that urinary 1-OHP is a byproduct of pyrene metabolism which occurs via the glutathione enzyme in the whole body, but is not due to changes in specific body cells or tissue.

2.7.6 Urinary 1-OHP Concentration and Gene Polymorphism

The genetic polymorphisms of enzymes normally affect the concentration of 1-OHP excreted with urine. Studies have also shown that these genetic polymorphisms of enzymes, including GSTM1, CYP1A1 and CYP1A1, also affect metabolisms associated with the formation 1-OHP (Jeng and Pan, 2015).

Even though the study by Petchpoung et al. (2011) demonstrated the relationship between exposure to PAHs and gene polymorphisms, the study did not investigate the relationship between urinary 1-OHP and the gene polymorphisms associated with controlling susceptibilty to diseases, as well as health complications caused by exposure to PAHs. Another such study by Sánchez-Guerra *et al.* (2012) in the Gulf of Mexico examined children living close to a petrochemical complex, and showed a positive relationship between levels of DNA damage and the level of 1-OHP found in their urine. This means that, according to Sánchez-Guerra *et al.* (2012), exposure to PAHs may cause DNA damage.

2.8 Literature Review

The section defines the basic framework and foundation of the study by reviewing the relevant theoretical and empirical literature, with the intention of understanding the effect of exposure to traffic-related polycyclic aromatic hydrocarbons (PAHs) on street janitors' health by quantifying the levels of urinary 1-hydroxypyrene (1-OHPyr) collected from their urine. This was executed using a thematic approach, in which the various aspects of the study were categorised into themes and sub themes, such as air pollution, contribution of transportation to emissions of air pollutants, Polycyclic Aromatic Hydrocarbons (PAHs), sources of PAHs, and urinary 1-hydroxypyrene as a biomarker for Benzo[a]Pyrene (BaP). A thematic approach was adopted because, according to Freud and Cronin (2013), it defines the theories, constructs and themes that are considered important in a study.

2.8.1 Literature Search Methods

According to the data available in Medline, PubMed, Google Scholar and a library search, 1,734 published articles from 1990–2019 have investigated 1-OHP as a biomarker in populations exposed to PAH agents in air. As shown in Figure 2-4 below, 1,734 articles reported on 1-OHP as biomarkers in populations exposed to PAHs agents in both animals and human beings. The abstracts of these articles were reviewed so as to select studies that meet the inclusion criteria. The procedure that was followed in selecting the most appropriate studies is shown in Figure 2-4 below. Some of the selected articles are summarised in Table 2-5. The keywords used during the literature search were: traffic-related air pollution, air pollution, biological markers, biomarkers, biological monitoring, biomonitoring, occupational exposure, Benzo[a]Pyrene, PAH, PAHs, urinary 1-hydroxypyrene (1-OHPyr) and pyrene.

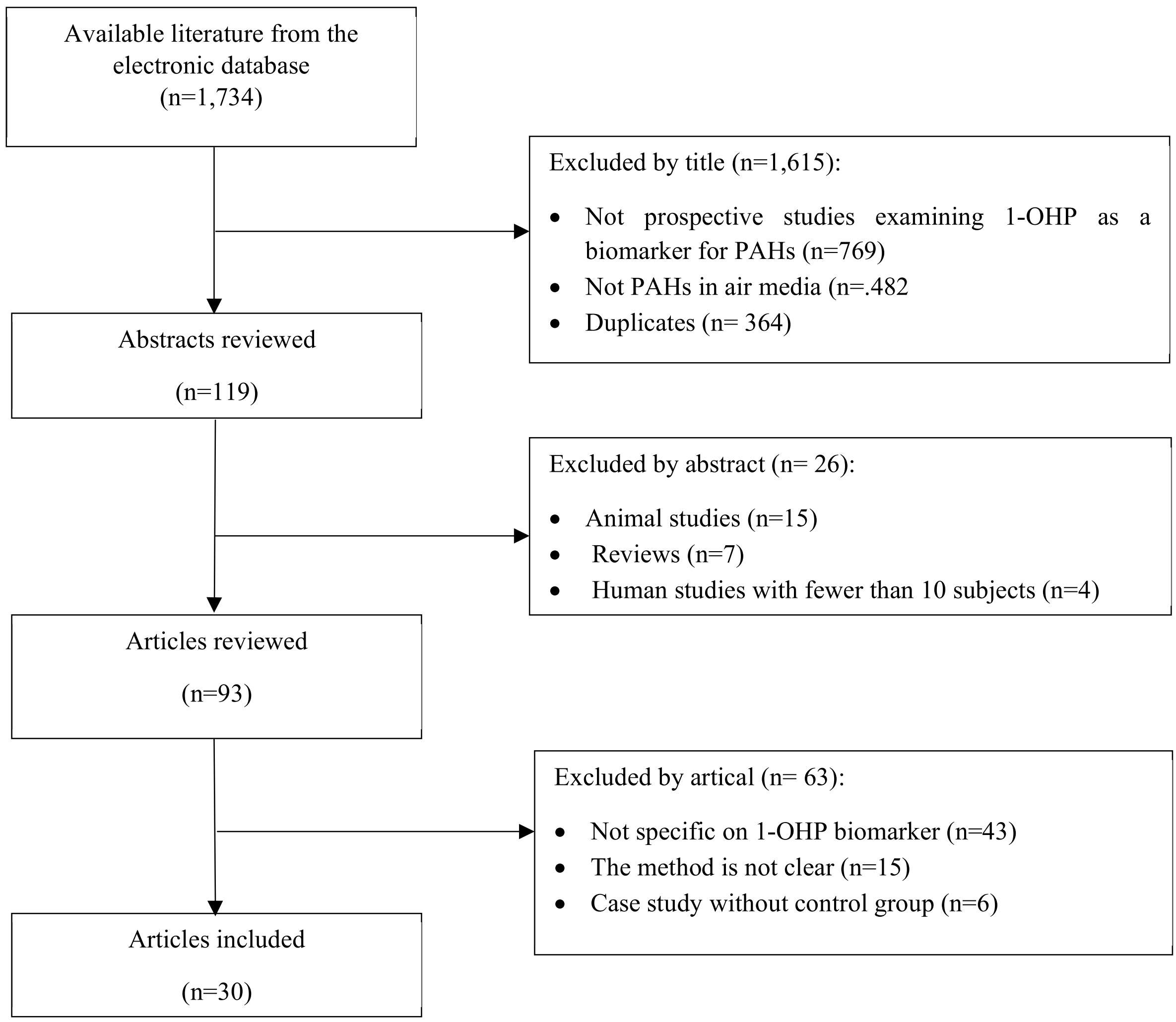


Figure 2-4: Literature review diagram

Table 2-5: Studies with B(a)P measurements in air and urinary 1-OHP biomarker

N	Author, year	Country	Type of PAHs sampled	PAH monitoring	PAH analysis technique	1-OHP analysis	Season of sampling	Subjects/participants	Number of subjects	Exposure
1	Zhao *et al.* (1992)	China	B(a)P	Personal	HPLC/FD	HPLC/FD	Winter + summer	Volunteer groups	65	Traffic
2	Kanoh *et al.* (1993)	Japan	B(a)P	n.a.	n.a.	HPLC/FD	Winter + summer	School children	82	Traffic
3	Hemminki *et al.* (1994)	Sweden	B(a)P	n.a.	n.a.	HPLC/FD	Winter	Bus and taxi drivers	70	Traffic
4	Ovrebø *et al.* (1995)	Poland	B(a)P	Personal	GC/MS	HPLC/FD	Winter + summer	Traffic police	66	Traffic
5	Gündel *et al.* (1996)	Germany	B(a)P	n.a.	n.a.	HPLC/FD	Winter + summer	Volunteer groups	124	Traffic
6	Černá *et al.* (1997)	Czech	B(a)P	n.a.	n.a.	HPLC/FD	Winter	Mail carrier + gardeners	187	Traffic
7	Gilbert and Viau (1997)	Canada	B(a)P	n.a.	n.a.	HPLC/FD	Summer	Volunteer groups	68	Traffic
8	Hara *et al.* (1997)	Japan	B(a)P	Personal	HPLC/FD	HPLC/FD	Summer	Garbage collectors	15	Traffic
9	Vyskocil *et al.* (1997)	Czech	B(a)P	Personal	HPLC/FD	HPLC/FD	Winter + summer	Volunteer groups	60	Traffic
10	Poirier *et al.* (1998)	Kuwait	B(a)P	Personal	GC/MS	HPLC/SFS	Summer	Army soldiers	61	Traffic
11	Merlo *et al.* (1998)	Italy	B(a)P	Personal	HPLC/FD	HPLC/FD	Summer	Traffic police	94	Traffic
12	Motykiewicz *et al.* (1998)	Poland	B(a)P	n.a.	n.a.	HPLC/FD	Summer	Volunteer groups	67	Traffic
13	Chuang *et al.* (1999)	USA	B(a)P	n.a.	n.a.	GC/MS	Winter + summer	School children	49	Traffic
14	Pastorelli *et al.* (1999)	Italy	B(a)P	Personal	HPLC/FD	HPLC/FD	Winter + summer	Office employees	65	Traffic
15	Siwińska *et al.* (1999)	Poland	B(a)P	Personal	HPLC/FD	HPLC/FD	Winter	School children	74	Traffic
16	Saint-Amour *et al.* (2000)	Canada	B(a)P	n.a.	n.a.	GC/MS	Winter	Volunteer groups	121	Traffic
17	Bouchard *et al.* (2001)	Canada	10 PAHs	n.a.	n.a.	GC/MS	Summer	Volunteer groups	30	Traffic
18	Staessen *et al.* (2001)	Belgium	B(a)P	n.a.	n.a.	HPLC/FD	Summer	Volunteer groups	200	Traffic

Note: Continued on the next page

Table 2-5: Continued

N	Author, year	Country	Type of PAHs sampled	PAH monitoring	PAH analysis technique	1-OHP analysis	Season of sampling	Subjects	Number of subjects	Exposure
19	Ruchirawat *et al.* (2002)	Thailand	PAHs	Personal	HPLC/FD	HPLC/FD	Winter + Spring	Traffic police	98	Traffic
20	Burgaz *et al.* (2002)	Turkey	B(a)P	n.a.	n.a.	HPLC/FD	Spring	Traffic police + Taxi drivers	32	Traffic
21	Hansen *et al.* (2004)	Denmark	B(a)P	n.a.	n.a.	HPLC/FD	Winter + Summer	Bus drivers + Mail carrier	87	Traffic
22	Chuang (2007)	Taiwan	B(a)P	Personal	GC/MS	HPLC/FD	Summer	Taxi Drivers	181	Traffic
23	Petchpoung *et al.* (2011)	Thailand	B(a)P	Personal	GC/MS	HPLC/FD	Winter + Summer	Bus Drivers	100	Traffic
24	Mcclean *et al.* (2012a)	USA	B(a)P	Personal	HPLC/FD	HPLC/FD	Summer	Paving Workers	144	Paving asphalt
25	Fan *et al.* (2012b)	China	B(a)P	n.a.	n.a.	HPLC/FD	Summer	School Children	39	Traffic
26	Huang *et al.* (2012)	Taiwan	B(a)P	Personal	GC/MS	HPLC/FD	Spring	Traffic Police	91	Traffic
27	Alghamdi *et al.* (2015c)	Saudi-Jeddah	B(a)P	Stationary	GC/MS	HPLC/FD	Winter	School Children	204	Traffic
28	Gong *et al.* (2015)	China	B(a)P	Stationary	GC/MS	HPLC/FD	Summer	Volunteer Groups	111	Traffic
29	Kamal *et al.* (2016)	Pakistan	B(a)P	n.a.	n.a.	HPLC/FD	Summer	Traffic police + Taxi drivers	122	Traffic
30	Samir *et al.* (2019)	Egypt	PAHs	n.a.	n.a.	HPLC	n.a.	Coke oven workers	85	Industrial exposure

HPLC, high performance liquid chromatography; FD, fluorescence detection; GC/MS, gas chromatography/mass spectrometry; SFS, synchronous fluorescence spectrometry. n.a., information not available.

2.8.2 Air Sampling Procedure and Analysis

Most studies carried out their air sampling procedure for the PAHs either by fixed site stations or a personal mobile air sampler. Fixed site stations are preferred when analysing ambient PAH conditions, but due to their fixed nature they are generally less flexible as they cannot be carried around. Another disadvantage is that they should be installed in locations where air is not restricted, and so such locations may not give the true conditions of the ambient air. However, these types of samplers are cheaper to maintain. Personal air samplers are flexible as sampling can be done anywhere and at any time although compared to fixed samplers, they are relatively expensive to maintain.

Of these two, the most dominant sampling technique is personal mobile air sampler as a result of its advantages. From the studies summarised in Table 2-5, it is observed that 13 studies (1, 4, 8–11, 14–15, 19, 22–24 and 26) used personal mobile air samplers, and only two utilised fixed site stations (27 and 28). The remaining studies did not mention the technique they used for air sampling. It is important to note that PAHs associated with particulate matter, such as Benzo[a]pyrene, are normally collected in filters (associated with these sampling procedures), vaporised from the filters, and then trapped by solid sorbent (Peltonen and Kuljukka, 1995).

Two major types of sampling techniques can be applied: passive sampling and active sampling. Active sampling involves the pumping of an air stream into the sampling device (Peltonen and Kuljukka, 1995), whereas passive sampling techniques depend on diffusion-controlled gradients to push air through the device (Peltonen and Kuljukka, 1995). Active air samplers do not depend on wind speed as air is pumped through the sampler, making them more reliable especially when wind speed speeds are extremely low. However, they need a power source to operate. Passive air samplers, on the other hand, depend on diffusion gradients and wind speeds.

When the speed of wind is low they become unreliable, but they do not require a power source, making them relatively cheap to operate.

2.8.3 Selection of Participants

1,734 studies were carried out on subjects at high risk of being exposed to airborne PAH pollulants by virtue of their profession or location in relation to the source of pollullants. These subjects included: coke-oven workers, school children, taxi and bus drivers, traffic police officers, and mail carriers. For instance, as shown in Table 2-5, five studies were conducted on school children (2, 13, 15, 25 and 27); three studies were conducted on bus drivers (3, 21, 23); four studies were conducted on taxi drivers (3, 20, 22 and 29); six studies were conducted on traffic police (4,1 1, 19–20, 26 and 29); two on mail carriers (6 and 21); two on paving workers (2 and 4); and one each on gardeners (6), coke oven workers (30), office employees (14), army soldiers (10), and garbage collectors (8). Nine were conducted on volunteer groups (1, 5, 7, 9, 16–18 and 28). However, it is not clear if these people lived near polluted districts or not. The selection of other subjects such as taxi drivers, garbage collectors, bus drivers, traffic police and mail carriers is logical since they are exposed to traffic-related air pollution during their working hours.

2.8.4 Sampling Procedure for Urinary 1-OHP and Analyses

During the literature review exercise, a discrepancy was noted in the time of the sample collection in many of the studies. There are studies in which samples were collected immediately after the exposure period (after work), while some were collected at bedtime and others early in the morning, hours after the exposure. For instance, in Mcclean *et al.* (2012a), the urine samples were collected at bedtime, post-shift, and pre-shift. The results of the study showed that bedtime and postshift concentrations of urinary biomarkers were significantly higher compared to the concentration of samples collected during the preshift. According to Alshaarawy *et al.* (2014), the appropriate time for collecting urine samples is the next early morning (pre-shift), as most metabolic reaction processes occur during the night hours, thus making the biomarker compound more concentrated than any other time of the day. Burgaz *et al.* (2002) noted that the lifespan of a biomarker in the human body ranges between 24–72 hours. Also, from Table 2-5, it can be observed that the most preferred method for analysing urine samples for 1-OHP is HPLC/FD, which was developed by (Jongeneelen *et al.*, 1990).

Two methods can be used to test urine samples for the presence of 1-OHP: HPLC/FD and GC/MS. From Table 2-5, it can be seen that eight studies (1, 8–9, 11, 14–15, 19 and 24) used HPLC/FD as the technique for analysis, while seven studies (4, 10, 22–23, and 26–28) used GC/MS as the method for analysis. In HPLC/FD, the analysis of the PAH samples uses high-performance liquid chromatography (HPLC) with a fluorescence detector. In GC/MS, the analysis is carried out using gas chromatography-mass spectrometry equipped with chemical ionization methods (electron and negative ionization methods) (Peltonen and Kuljukka, 1995). It is important to note that, regardless of the fact that the methods may be different, they often yield a similar result. However, studies have shown that the GC/MS technique cannot resolve isomeric structures. Because of this, HPLC has been the detection technique for many researchers (Peltonen and Kuljukka, 1995).

2.8.5 Effect of Seasons on the Accumulation of Airborne Pollutants

The four seasons often play a significant role in the amount of airborne pollutants available in the air. For instance, Pastorelli *et al.* (1999) proved that the concentrations of air pollutant compounds tends to increase during the winter compared to other seasons. They attributed this to an increase in the air density during the winter season, slow wind movement, and the low temperature. These conditions, according to the researchers, tend to maintain the chemical compounds for long periods of time before they disintegrate. It is for this reason that most of the studies shown in Table 2-5 were conducted during the winter season. According to Table 2-5, it is noticeable that not many studies have been conducted throughout the whole year to include the four seasons. Therefore, the application of the current study over a year will give a clearer picture of the pattern of pollution during the year, and the impact of the four seasons on it.

2.8.6 The Study of Exposure to PAHs in Saudi Arabia

A number of studies in Saudi Arabia have investigated exposure to PAHs and associated health complications. For instance, Alghamdi *et al.* (2015c) conducted a study to determine the relationship between exposure to PAHs and the concentration of hydroxyphenanthrenes and 1-hydroxypyrene in urine samples in Jeddah, Saudi Arabia. Ali *et al.* (2016) studied indoor exposure to PAHs in Jeddah City and Kuwait, finding high concentrations of PAHs in indoor microenvironments in Jeddah. Shabbaj *et al.* (2018) studied human exposure to PAHs in Saudi coastal cities and the health risks associated with this exposure. The study involved collecting street dust samples in five types of area: urban, traffic, residential, suburban and mixed residential and commercial areas in Jeddah City. The study also collected samples in Hada Al Sham, which is a rural area. The results showed that the concentration of PAHs in Jeddah City is almost 15 times more compared to PAH concentrations in Hada Al Sham. This is mainly because Jeddah is the second largest city in Saudi Arabia, and has high traffic volumes and more industry compared to Hada Al Sham.

2.8.7 PAH Exposure in Occupational Settings

1. **High PAHs Exposure**

As chemical carcinogens, PAHs rank as the ninth most threatening compounds to human health (Šimko, 2002), and comprise the largest class of chemical compounds causing cancer. Incomplete combustion of organic materials, such as coal, oil and wood, are main sources of PAH emissions (Kim *et al.*, 2014a). Industrial activities where exposure to PAHs is high include coke ovens, coal tar use, iron and steel works, aluminium works, foundries, and asphalt manufacture. Inhalation and dermal absorpation of volatile compounds / or PAHs associated particles are important routes of exposures (Unwin *et al.*, 2006). Total concentrations of PAH in the air are highly variable, especially within the industrial sectors, and are dependent on emission sources and various exposure determinants, such as the composition of the product, process type and temperature, ventilation, and confinement (Lutier *et al.*, 2016; Maitre *et al.*, 2018).

PAHs concentrations in highly contaminated occupational settings (e.g. oil industry and paving sector) have been measured by many investigators. Xu et al. (2018) measured PAH metabolites, 1-hydroxypyrene (1-OH-PYR) and 2-hydroxyphenanthrene (2-OH-PH) in urine and relative mtDNAcn and telomere lengthamong 116 workers paving conventional asphalt, 51 workers paving crumb rubber modified (CRM) asphalt and 100 controls in Sweden, all males. The results showed that both conventional and CRM asphalt workers had higher 1-OH-PYR and 2-OH-PH than control group ($p < 0.001$ for all) and the relative mtDNAcn were 0.21 units ($p < 0.001$) higher in the conventional asphalt workers, and 0.13 units ($p = 0.010$) higher in CRM asphalt workers. Two limitations were indicated by the authors in their comparative study of a small number of workers paving with CRM asphalt or conventional asphalt; their limitations restrict the possibility of determining differences in exposure and effects. In addition, the levels of air-borne nitrosamines and dust to which the workers were co-exposed were not estimated due to outdoor work complexity and large variations of asphalt mix and paving temperature.

Mcclean *et al.* (2012a) studied the extent of exposure to polycyclic aromatic compounds (PACs) (e.g. 1-OH-pyrene; 1-, 2-, 3-, 4-OH-phenanthrene; 1-, 2-OH-naphthalene; 2-, 3-, 9-OH-fluorene) among workers working with hot asphalt mix by analysing urine (n= 480); hand wash (n= 144) and personal air (n=480) samples. The urine samples were collected at preshift, postshift and bedtime and analyzed by an immunochemical quantification of PACs (I-PACs), using a different exposure scenario: a baseline week (normal conditions), a dermal protection week (protective clothing), a powered air-purifying respirator (PAPR) week, and a biodiesel substitution week (100% biodiesel provided to replace the diesel oil normally used by workers to clean tools and equipment). The results showed that the postshift and bedtime PAC-concentrations were significantly higher than preshift ones for most urinary biomarkers. In addition, compared with baseline, urinary analytes were reduced during the dermal protection (29% for 1-OH-pyrene, 15% for I-PACs), the PAPR (24% for 1-OH-pyrene, 15% for I-PACs), and the biodiesel substitution (15% for 1-OH-pyrene). Moreover a positive linear correlation was found between temperature of hot-mix asphalt and urinary PAH metabolites concentrations, so reducing temperature of asphalt mix is a promising strategy to reduce exposures. The question arising here is to what extent the range of application temperatures can be evaluated and the extent to which further reductions in exposure can be achieved, without affecting the quality of asphalt application.

Firefighters are heavily exposed to carcinogenic PAHs released from fires. Stec *et al.* (2018) investigated the exposure of firefighters to PAH carcinogens. 1-OHP was analysed in wipe samples from skin (jaw, neck, hands), personal protective equipment, and work environment (offices, fire stations and engines) in two UK Fire and Rescue Service Stations.The results showed evidence for presence of PAH carcinogens (e.g. BaP, 7,12-dimethylbenz (a) anthracene and 3

MCA) on wipe and work environment samples, indicating that the firefighters are at risk of exposure to PAHs. PAHs are linked to certain cancers, but it has not been proven that these agents are causative as there is insufficient epidemiological/experimental data on individual PAHs and mixtures. It is worth mentioning that it is both important to determine the exposure levels of other combustion-generated contaminants, and the effects of such exposures either singly or as mixtures (Llabjani *et al.*, 2010). Furthermore, long-term and detailed follow-up studies are required to capture individual PAH exposures in order to determine if they are responsible for the elevated cancer incidence in firefighters (Stec *et al.*, 2018), and to look for chromosomal aberrations in erythrocytes or bulky chemical-DNA adducts in lymphocytes or the effects on circulating free DNA (Hewitt *et al*., 2007). Workers of coke facilities are exposed to PAH and volatile organic compound emission in work environment (Tripathi *et al.*, 2016). Coke oven workplaces had higher levels of carcinogenic compounds than other workplaces (Bieniek and Łusiak, 2012), and significant associations were found between PAHs exposures with urinary hydroxypyrene, DNA-PAH adducts, total nitro-PAH hemoglobin adducts and sister chromatid exchanges (Vimercati *et al.* (2020). The occupational exposure to PAHs was thought to be responsible for 188- lung cancer cases, among exposed male workers, in France in 2000 (Thun *et al.*, 2008), and for 131 (95% CI: 5–266), in Italy in 2005 (De Matteis *et al.*, 2012). In fact, the difference in exposure level and length can result in different responses in the body (Xu *et al.*, 2018). No correlation was found between changes in PAH metabolites and post-working mtDNAcn among all asphalt workers. However, an increase in 1-OHP with a decrease in mtDNAcn was observed in 31 workers, and can be attributed to the unmeasured nitrosamines or other co-exposures such as traffic pollutions, that were expected to be responsible for the increased mtDNAcn. The dose-response of PAH exposures should be estimated, as compounds other than PAHs or unmeasured co-exposures (e.g. traffic pollution outdoor work complexity, and large variations of asphalt mix) may be responsible for increasing PAHs levels (Xu *et al.*, 2018). Moreover socio-economic factors and the social behaviour of the workers may be associated with their vulnerability, but further investigation is needed.

2. **Low PAHs Exposure**

Many of previous studies have been conducted in industrial cohort where PAH concentrations are high. However little attention is paid to determine PAH concentrations in general population/ or occupants working in city streets, where PAH concentrations are low. PAHs are ubiquitous in the global environment and are typically more concentrated near urban centers (Hyötyläinen and Oikari, 2004), due to the density of the population, vehicular traffic, and poor dispersion of atmospheric pollutants in street canyons and the built environment.

Ifegwu *et al.* (2012) attempt to assess the level of this biomarker in the body fluid of 68 exposed subjects using high performance liquid chromatography HPLC. The subjects screened included auto mechanics, drivers, and fuel attendants. 1-hydroxypyrene was extracted from the urine of the subjects using solid phase extraction method. They determined biomarker,1-OHP levels, in urine of 68 exposed subjects, including auto-mechanics, drivers and fuel attendants using high performance liquid chromatography (HPLC). The results showed that ~ 27% of sampled fuel attendants and 22% of auto mechanics had detectable 1-OHP in their urine samples, and there was no detectable 1-OHP in the urine samples of commercial drivers or students (control group).

In addition, Leem *et al.* (2005) found higher levels of urinary 1-OHP in the taxi drivers compared to participants exposed to low levels PAHs (passengers). Fang *et al.* (2004) indicated that mean of total PAHs levels at industrial, urban, and rural areas in central Taiwan region ranged from 1,232 - 1,650; 700 - 1,740; and 610 - 831ng/m^3, respectively. Unwin *et al.* (2006) found that the total weight average of PAH concentrations in the air in UK$^-$ industrial areas ranged from 0.4 –1,912.6 µg/ m^3, with naphthalene was the dominant PAH compound and benzo(a)pyrene concentration was associated with concentration of the carcinogens 4–6 PAHs. Currently, limit values of 1 or 10 ng benzo(a)pyrene /m^3 air are recommended or mandatory in various countries (Italy & Germany), and nowadays, these concentrations are seldom found or exceeded in the ambient air of rural or even of urban living areas (Jacob and Seidel, 2002). So far, no established standards have been set for the level of PAHs in the urine of the general population and more specifically for the pedestrians.

3. PAHs Exposure in Traffic-related Occupations

Personal PAH exposure due to urban traffic has been the subject of several investigations, in general population (Minoia *et al.*, 1997) or in occupationally exposed individuals, such as policemen (Perico *et al.*, 2001), and newsagents (Piccardo *et al.*, 2003). Evaluating the daily personal exposure to benzo(a)pyrene is one of the most important of taxi drivers (Menichini, 2015). Kamal et al. (2016) investigated the effect of traffic related PAHs exposure on blood of traffic police officers, drivers, and office workers (a control group). PAH-biomarkers (urinary 1-OHP, β-naphthol and α-naphthol) were measured and compared with inflammation biomarkers (WBCs & CRPs), DNA damage (8-hydroxy-2-deoxyguanosine), and oxidative stress. The results showed that the concentrations of PAHs exposure biomarkers and inflammation biomarkers (CRPs and WBC) were significantly higher between traffic police officers than the other groups under unvestigation. Moreover the urinary biomarker for the oxidative DNA damage (8-OHdG level) was higher among the police officers than drivers and office workers. This indicates that people working in traffic-related occupations are exposed to higher PAHs levels and they are at increased risk (Kamal *et al.* (2016).

Gong *et al*. (2015) studied traffic related PAHs exposure of 111 smoking and non-smoking participants during and after the Beijing Olympics. The urine samples were analysed to determine the presence of 1,2-amino-naphthalene and 1-amino-pyrene. The results showed that the concentrations of 1-OHP were related to the level of traffic pollutants, as traffic air pollutants were mainly associated with PAHs. Therefore, people who interact with traffic on a regular basis, for example, traffic police officers, and drivers and their passengers, may be exposed to high levels of organic compounds Surprisingly, a comparison between subway and car exposures in Beijing showed significantly higher concentration of PAHs in the subway train (Srogi, 2007).

Petchpoung *et al.* (2011) found a significant higher concentration of 1-OHP among the Thai bus drivers compared to the general population, in addition to a difference in 1-OHP concentrations between smoking and non-smoking of bus drivers, and the elevated levels of 1-OHP was related with CYP1A1 and the exon 7 variants, but drivers with GSTP1 Val and GSTM1 null genotypes showed decreased excretion of urinary 1-OHP. Moreover, no relationship was found between GSTT1 polymorphisms and urinary 1-OHP concentration among the bus drivers.

In fact, cigarette smoking and air pollution are the main sources of PAH exposure, and may cause polymorphism of GSTM1, GSTP1 and CYP1A1 genes. Chuang (2007) evaluated concentrations of urinary 1-OHP between 95 taxi drivers and 75 office workers, to assess the relationship between traffic exposure, lifestyle, metabolic enzyme polymorphisms and the concentration of urinary 1-OHP. The results showed that urinary 1-OHP concentrations were higher in the taxi drivers than the office workers, indicating that traffic exhaust exposure, smoking and CYP1A1 Mspl genotype affect variation in 1-OHP excretion levels, and that working in traffic or traffic- congested environments increase the risk of exposure to PAHs.

Hansen *et al.* (2004) evaluated the concentration of 1-OHP and gene mutations in urine of bus drivers and mail carriers in Danish cities and urban areas. The results showed that bus drivers were highly exposed to PAHs than the mail carriers, and the mail carriers working outdoors were more exposed to PAHs than those working indoors. However no-relationship was found between exposure to PAHs and gene mutations, because concentration of urinary 1-OHP was not -related to concentration of urinary mutagenic activity, as most of the mutagenic compounds in diesel are not PAHs but dinitro-pyrenes. Burgaz *et al.* (2002) studied the cytogenetic effects of PAHs exposure among 17 taxi drivers, 15 traffic police, and 23 healthy men (control group), in Ankara, Turkey. The study found that traffic police officers and taxi drivers had higher urinary 1-OHP concentrations and chromosomal aberration levels than the comparison group indicating that urban air, especially around traffic is associated with PAH pollutants,and increased the risk of cytogenetic effects. The exposure of Thai's non smoking-traffic police officers to PAHs associated particulate matter in ambient air was studied (Ruchirawat *et al.* (2002). The results showed that traffic police officers were ≥20 time folds exposed to PAHs compared to those working in offices, and police officer who spend most of the time outdoors had a higher chance of being exposed to PAHs than who tend to stay indoors.

Biomonitoring studies that showed urinary PAH metabolite levels, as well as demographic and lifestyle information, are used to find the relationships between PAH exposure and variables such as smoking status, age, sex, and occupation *inter alia*, as shown by Keir *et al.* (2020). The authors surveyed creatinine-adjusted urinary PAH metabolite concentrations in Canadian individuals aged 16 years and over, and found that smoking status, age, and sex are associated with urinary concentrations of a pyrene metabolite (1-OHP), phenanthrene metabolites (ΣOH-Phen), fluorene metabolites (ΣOH-Flu) and naphthalene metabolites (ΣOH-Nap). Recently, studies on PAHs in particulate matter (PM), such as PM_{10} and $PM_{2.5}$ in ambient air, have attracted the attention of many researchers (Byeong-Kyu and Van-Tuan, 2010). The toxicity of particulate matter from different sources is a public health threat that includes a wide range of chemical compositions with different bioaerosol and concentrations in PAH mixtures varying with different seasons. Guo *et al.* (2018) studied the concentration and size distribution dynamics of PM in Beijing during a red alert air pollution event in hazardous and unhealthy haze days compared to sunny days in 2016. Results indicated that the PM concentrations on the haze days were, respectively, 10.7 and 8.0 times higher.

4. PAHs Exposure in Non-Occupational activities

PAHs exposures may be associated with occupational and non occupational activities. Naturally people may be exposed to PAHs in relatively clean atmospheres. Alghamdi *et al.* (2015c) determined the relationship between exposure to PAHs (pyrene and phenanthrene) from dietary and atmospheric souces and the concentration of hydroxyphenanthrenes and 1-hydroxypyrene in 3 consecutive urine samples of 204 male school children in Jeddah, Saudi Arabia. The results showed that the increase in ambient air pyrene and phenanthrene concentrations was related to the increase in the concentration of 1-hydroxypyrene and hydroxyphenanthrene in urine, respectively. Alghamdi and his colleagues concluded that it is not only the exposure to environmental pollution that causes exposure to PAHs, but also consumption of certain food types, such as grilled food which increases the concentration of hydroxyphenanthrene and 1-hydroxypyrene in urine samples, ending to a conclusionn that dietary intake is the main unexplained variable and the major route of PAH exposure. Basic information about food choices and consumption were mainly surveyed using questionnaires. The accuracy of such questionnaires is known to be hindered by significant reporting biases and errors, with individuals often under-reporting food consumption, sometimes at implausibly low intake levels compared with estimated energy needs (Cook *et al.*, 2000; Bedard *et al.*, 2004).

Mucha *et al.* (2005) evaluated the PAH exposure among 48- three-years-old children who lived in Mariupol, Ukraine near a steel and coke mill and 42 children of the same age in Kiev, the Ukrainian capital. The reuslts showed that children living nearby the large industrial areas are exposed to high levels of PAH pollutants with about 50% higher 1-OHP concentration (0.69 μmol/mol) comapred to the capital city children with 0.34 μmol/mol of 1-OHP.

Furthermore, Fan *et al.* (2012b) investigated the association between levels of PAH metabolites in urine and 8-OHdG (which is a biomarker for DNA damage) of children exposed to traffic-related polluted air. The samples were collected from two participant groups: children in an elementary school located near a heavy traffic road of Guangzhou, China, and children in a kindergarten located inside a large university, less or unpolluted. The study found a higher concentration of PAH metabolites (2-hydroxynaphthalen, 2-hydroxyfluorene, 3- and 9-hydroxyphenanthrene, and 1-hydroxypyrene) in the urine samples collected from the group of students in the location near the heavy traffic road, as heavy traffic pollution was the main cause of high concentration of PAHs. However no significant relationship was found between the exposure to PAHs and DNA damage, and no significant difference was also found between the two groups, regarding levels of 8-OHdG in the urine, but children in the elementary school located near a heavy traffic road had slightly higher concentrations of 8-OHdG in their urine than the comparison group.

In fact, most of the reviewed papers were missing an evaluation of exposure uncertainties. ‘Uncertainty’ is sometimes defined as all possible sources that challenge the validity of a study and the aim of its evaluation is to be able to make reliable decisions. Different sources of exposure may result in various types of error that should be considered to minimize their effects on risk estimates (Wu *et al.*, 2019).

It can be said that epidemiological studies of environmental exposure should include exposure-response analyses which account for uncertainties in exposure estimates. However, only a few publications have mentioned that ‘measurement error’ or ‘uncertainties’ may exist in exposure assessments, and even fewer have assessed the effect of measurement and/or estimation error on risk estimates. Failure to account for uncertainties in exposure estimation may lead to biased results and undue confidence in the accuracy of the subsequent exposure-response analyses.

Exposure uncertainties may be evaluated based on the researcher's knowledge of the type of distribution of each parameter (Hofer, 2008). However, variability in exposure cannot be considered a type of uncertainty, as this variability reflects the inherent heterogeneity of exposure across individuals which will still exist due to randomness, even if all other exposure characteristics (such as sex, age, lifestyle, location of residence, diet, job identifiers, etc.) are identical across the set of individuals.

In practice, the error of exposure estimation is usually complex and contains various types of error, such as shared and unshared random errors as well as unshared non-random errors, although one type usually predominates. In such cases, more advanced statistical approaches are needed to account for the complex error structure in risk analyses, for example, the widely used Monte Carlo methods and the most advanced two-dimensional Monte Carlo approach. This is a simulation-based exposure reconstruction strategy that makes multiple realizations of possibly true exposure estimate alternatives, and allows researchers to use various statistical approaches to account for shared and unshared sources of uncertainties in exposure-response analyses (Stayner *et al.*, 2007).

Epidemiological textbooks state that random errors in exposure estimation lead to the attenuation of the exposure-response relationship. There are four main statistical methods to account for effects of errors in exposure estimation on risk estimates: regression calibration, simulation-extrapolation, Monte Carlo maximum likelihood, and Bayesian model averaging. Hence, comprehensive evaluation of potential error structures in the exposure estimates is important when developing an exposure estimation protocol because it can lead to an improved exposure-response relationship by eliminating biases that can occur when uncertainties are underestimated. Thus, expectations indicate that, after accounting for exposure estimation uncertainties, risk estimates should increase.

2.8.8 Islamic Calendar

The Islamic calendar is also called the Hijri calendar and is calculated according to the occasion of the migration of the Prophet Muhammad from Mecca to Madinah and the beginning of the emergence of Islam (Paul, 2005). It is a 12-month lunar calendar in a year of 354 or 355 days (Sharjah, 2019). Muslims use it to identify religious occasions and rituals such as the period of fasting (the month of Ramadan) and performance of the rituals of Hajj (Watt, 2019). It is an official calendar for most Arab and Islamic governments, especially in Saudi Arabia (Sharjah, 2019). Each month of the Islamic calendar begins at the birth of the new lunar cycle, and thus each month can have 29 or 30 days depending on the moon's visibility, the Earth's astronomical location, and weather conditions (Watt, 2019).

The Hijri calendar begins with the month of Muharram (month one) and it is considered mid-month within the months of the rituals of Hajj, followed by the month of Safar (month two), which is a normal month when there are no religious events. The other normal months after that are during the month of Rabi al-awwal (month three), Rabi ath-thani (month four) Jumada al-ula (month five), Jumada al-akhirah (month six), Rajab (month seven) and Sha'ban (month eight). After that, Ramadan (month nine) is the holy month of fasting for Muslims, and in which worship, prayer and collective breakfast take place. Then comes the month of Shawwal (month ten), which is another normal month after the month of fasting. The last two months in the Islamic year are Dhu al-Qadah (month 11) and Dhu al-Ḥijjah (month 12), when Muslims perform the rituals of Hajj and worship (Watt, 2019).

This study was designed according to the Islamic calendar year due to its implementation in Madinah, where religious events directly have an effect. Therefore, the results can be interpreted according to the variables that occur as a result of religious events associated with the Islamic calendar for the whole year.

2.8.9 Study Gaps

From the above literature, it is obvious that there are many gaps in the field of knowledge. Five research gaps were selected to be addressed in this research. The first is regarding the city of study. From Table 2-5, it is obvious that no study has been conducted in Madinah, Saudi Arabia. This is regardless of the fact that Madinah plays an important role in the Muslim world with regards to the number of visitors coming to the city every year. This city was therefore chosen in order to evaluate how such religious events can affect air quality in terms of exposure to PAHs (BaP to be specific). The second gap regards the measurement method of BaP. From Table 2-5, it is clear that most studies relied on one measurement method, but this study applied two different methods to increase the confidence in the results. The third gap is that most studies were conducted during sporadic days of the year or according to the seasons (Table 2-5), while this study was conducted over the whole year, including four seasons. The fourth gap concerns the measurement of urinary 1-OHP samples. Most studies did not measure urinary 1-OHP more than once to confirm the quality of the results. Some researchers collected only one sample, which makes the result weak. Most studies also relied on taking urine samples on different days of the year (not continuous days). However, this study sampled urine from the participants twice (once after work and the second the next morning), continuously for one year. The fifth and final gap is concerned with the category of participants. From Table 2-5, it can be observed that most of the individuals in previous studies were traffic police officers, drivers, school children, fire officers, road pavers, mail workers, gardeners, volunteers, and office workers. No research has studied the extent of exposure of council workers (especially those working outdoors, such as street janitors) to PAHs. This is regardless of the fact that such outdoor council workers work in areas that are heavily polluted with traffic, and spend most of their time in these polluted environments. The current study, therefore, evaluates the extent to which street janitors are exposed to BaP in the course of their daily activities.

CHAPTER THREE

Materials and Methods

Chapter 3: Materials and Methods

3.1. Introduction

This chapter presents the design of the study, including the city where the study was carried out and the specific locations where data were collected, as well as the rationale for their selection, the period of data collection, and the sample of people under investigation. In addition, the chapter presents the equipment for sampling, the methods of sample analysis, and the statistical treatment of the obtained data.

The criteria for selecting the city of Madinah are based on its religious importance and the fluctuations in the density of population, and accordingly in traffic density and types of vehicles. Moreover, the selection of city janitors relates to the lack of literature and data concerning this group, despite how they are directly affected by environmental contaminants due to the nature of their work. In addition, this chapter includes the scheme used to achieve the objectives of the study, and the data correlations and meteorological measurements supporting the obtained data.

3.2. Study Design

Madinah city, Saudi Arabia have been chosen for the study due to its religious importance. The location of Madinah relative to other Saudi Arabia cities is shown in Figure 3-5, while the major streets connecting the outskirts of Madinah and the grand mosque (Haram) is shown in Figure 3-6. Ethical approvals were obtained from Newcastle University (Appendix 7.3) and Madinah Municipality (Appendix 7.1). Informed written consents were obtained at the beginning of the study from all participants (Appendix 7.5).

The study began on 1/4/1438 to 30/3/1439 (corresponding to the Gregorian calendar dates 30 December, 2016 to 18 December, 2017). Metrological measurements were collected daily for 354 days during the study year and samples (air and urine) were collected only on days when the participants were working (20 days per month).

Figure 3-5: Google map showing the location of Madinah relative to other cities in Saudi Arabia (Mecca & Riyadh). *Source: Google Maps (2019)*

Figure 3-6: A satellite image for the major streets of Madinah, Saudi Arabia. *Source: Google Maps (2019)*

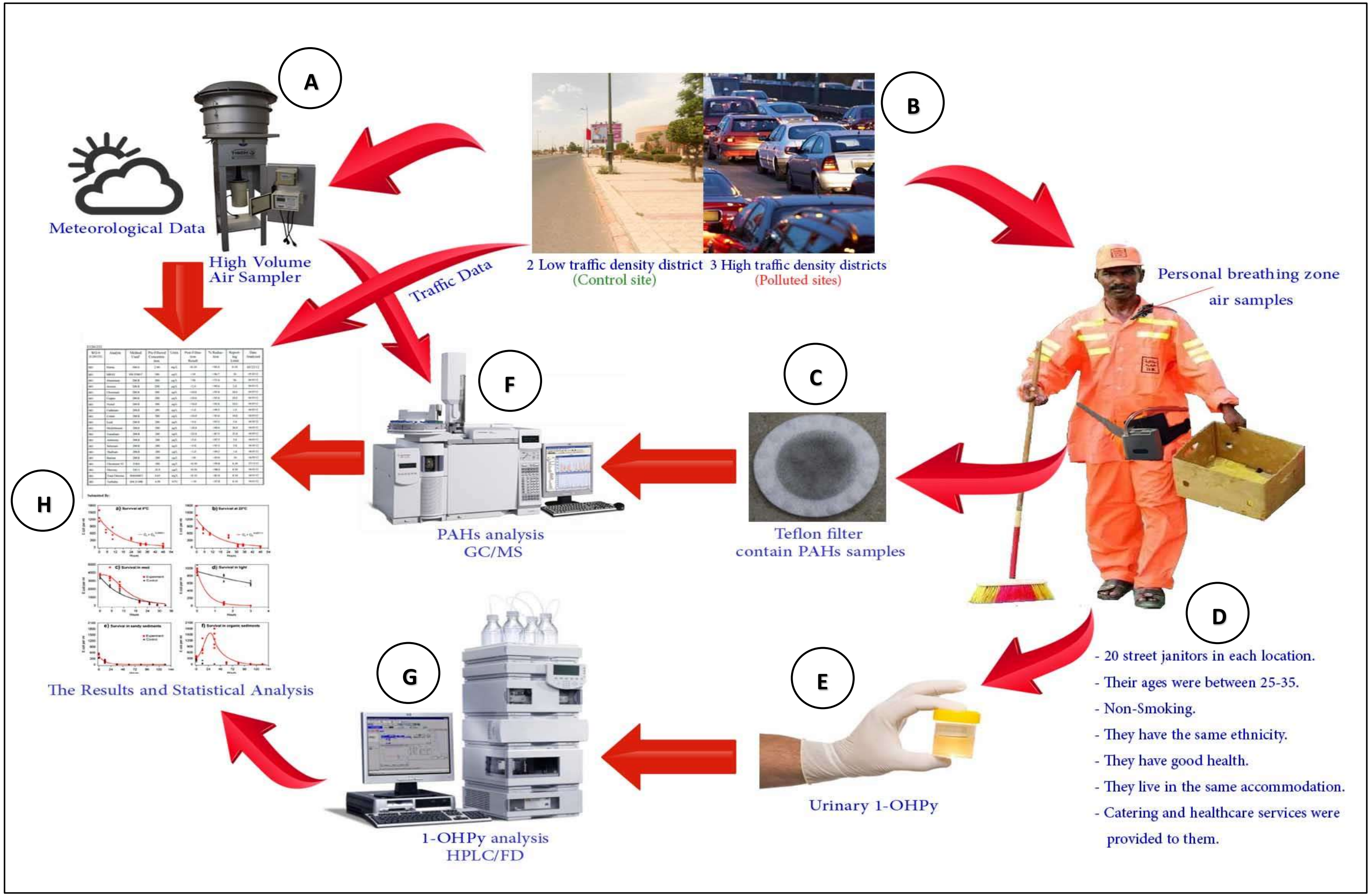


Figure 3-7: Flow chart of the study design and methods *Source: Author's photos and design*

3.3. Methods of Sample Collection and Analysis

Figure 3-7 illustrates schematic diagrams for the high volume air sampler: (A) with polyurethane foam (PUF) filters used to collect ambient air samples from the five locations under study (three traffic and two control locations); (B) the personal breathing-zone air sampler with Teflon filter; (C) the connection of a pump around the waist of each participant via a silicon tube used to collect personal air samples in the breathing zone of street janitors distributed in the five locations. The selection criteria of the street janitors (D) included in the study are also mentioned. Urine samples (E) from participants to determine the 1-OHP resulting from exposure to air-contaminant BaP were also illustrated. All samples were sent to the laboratory for analysis. Ambient and personal BaP concentrations were determined by GC/MS (F), while 1-OHP in urine was determined by HPLC/FD (G), followed by statistical analysis of data (H). In addition, daily records for traffic and meteorological variables were also collected.

3.4. Selection of Study Locations

The locations included in the study were three crowded and heavy-traffic density locations (Downtown, Quba and Alwali) and two low traffic-density control locations (Al-Azizyah and Sultana), as shown in Figure 3-8. For the purpose of selecting the locations for this study, data traffic records were reviewed from the Healthy Cities Programme in Madinah (HCPM, 2015).

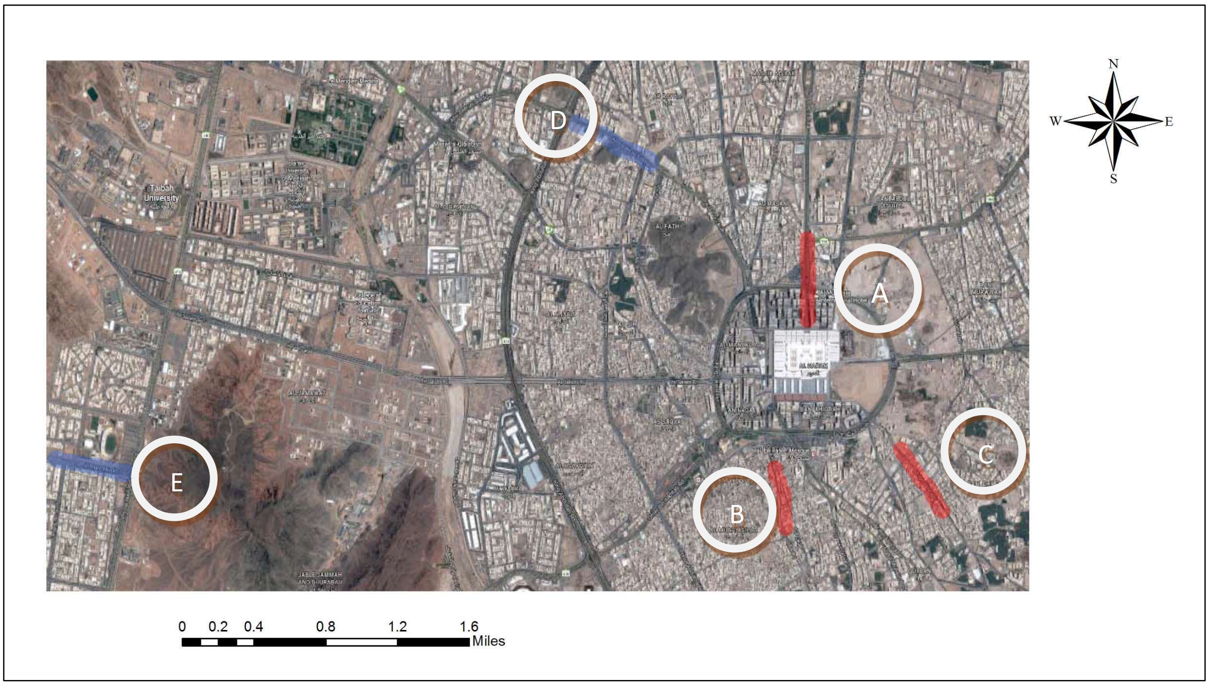


Figure 3-8: The Study Locations: (A) Downtown, (B) Quba, (C) Awali, (D) Sultana, (E) Al-Aziziyah. *Source: Google Maps (2019)*

Location A is Downtown, where the Prophet's Mosque is located and there are mostly crowded narrow streets, with a high population density. Heavy traffic density is observed especially in the holy months due to the huge number of diesel engine buses transporting pilgrims and visitors to and from this location. Location B is Quba, between the Prophet's Mosque and Quba Mosque. It is one of the most important commercial locations and is very densely populated. Location C is Awali Street, also characterized by a high density of vistors and residents. The commercial street in Awali is very narrow and is one of the most crowded streets, as it links the south of the city to the city centre. Location D is Sultana Street, which is a highly-urbanized area with a large number of markets and cafés. The general traffic flow is low due to traffic barriers and many pedestrian paths. Location E is Al-Aziziyah Street, which is distant from pollution sources. It is a model residential neighbourhood with no crowded streets and a low population density.

3.5. Selection of Study Participants

The Madinah Municipality recruits and contracts workers (Figure 3-9) for sanitation and hygiene. There are 3,000 workers patrolling the streets of Madinah during 8-hour work shifts (from 3pm to 11pm). All the workers work a five-day week and have two days off. However, the participants for this study worked for three consecutive days during the data collection without a break, to ensure that the most accurate results were obtained when examining the urine samples for 1-OHP. The participants consisted of 20 street janitors in each of the five study locations, making a total of 100. Aged between 25–35, they were in good health according to their medical reports (Appendix 7.12). Additionally, they were non-smokers, of the same ethnicity, and living in the same accommodation. Catering and healthcare services were provided to them during the study period. It is, however, important to note that meals, accommodation and healthcare were the same (standard) for all the workers, not just the participants.

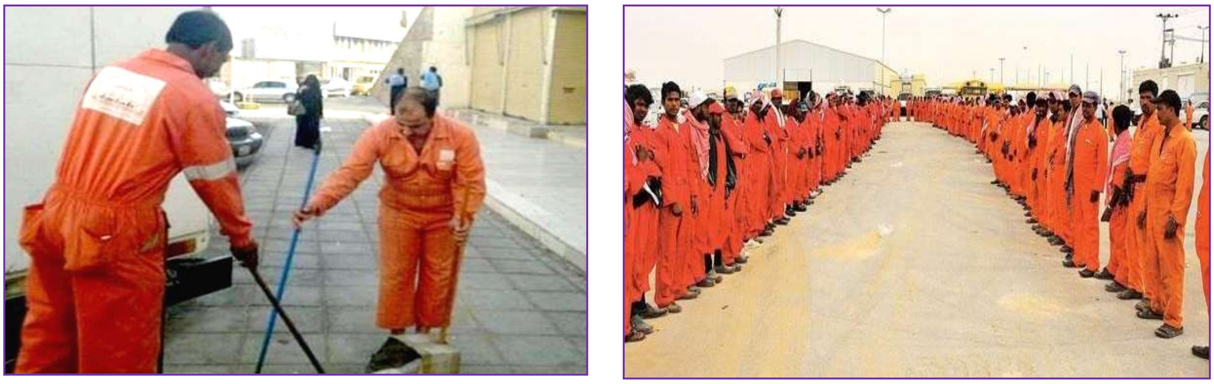


Figure 3-9: Street janitors belonging to the Madinah Municipality *Source: Author's photos*

The present study focused on this group of people as they are directly exposed to outdoor air pollution and traffic emissions. The Madinah Municipality contracts them to clean the city and remove solid waste for recycling. Through their contract, they are provided with accommodation, catering, health care, a sports centre, and transportation. They live on the company's campus, which consists of ground floor lines made of concrete, located five miles from the motorway. Four participants shared a 5m x 5m room with a window, an air conditioner for good ventilation, electricity, and a bunk bed. The site has green spaces, no internal toilets or indoor kitchens, and no vehicles are allowed to enter the campus. Workers were not allowed to use any incense to avoid any external sources of pollution. Regarding catering, the participants were told not to cook their meals or to have food from outside the campus. They were provided with three meals per day, including breakfast, lunch and dinner, of all food varieties, but no grilled food was allowed. There was a dining hall with no direct access to the kitchen. A coach was available to transfer them in 20 minutes to the work area, about 15 miles from the campus. To ensure that participants were not subjected to extra pollutants during their work period, the coach used diesel fuel. Its capacity was 55 workers, and there was no traffic density during the round trip. In addition, workers were not allowed to use any other transportation to reach their work areas (Appendix 7.4).

After selection, participants were subjected to intensive medical examinations (medical reports are attached in Appendix 7.12), to find out the general health status for the selected participants and ensure that they do not have any health problems that require their exclusion in order not to positively or negatively bias the results of the study. Some medical examinations (Cardiovascular, Respiratory, Gastro-intestinal and Venereal diseases) as well as laboratory investigations (urine and blood) were conducted two weeks prior to the start of the study. All the selection criteria of the personal traits and the health examination results ensured the similarity between the participants regarding their age, personal traits and health status. In addition, the urine sample collection exercise was designed such that samples are withdrawn from twenty participants from the same location and work shift. This minimized the potential for large variation (difference) in the properties of urine samples (in terms of 1-OHP concentration) collected from different participants in the same locality. It is important to note that the aim of the research was to study variation of 1-OHP concentration by place (traffic and non-traffic areas) and not the variation of 1-OHP concentration by participant, for that reason, it was necessary to integrate the most common and important factors such as accommodation, work location and living environment.

3.6. Samples Collection

Figure 3-10 represents a schematic diagram of one of the study locations for the places where the measuring equipment was placed, and the participants, samples and data collection procedures. In (A) was can see a selected street janitor carrying the personal air sampler device during their 8-hour work shift patrolling a two-way street (D) of the traffic or control locations, each in the working location. Participants were asked to move within 0.2 miles to be in the same area of the high-volume air sampler (B), located inside a frame on the edge of the side pavement and operated to collect samples five days per week. In the same place as the ambient air sampler, there was a digital video device (Miovision Scout system) for capturing traffic and counting cars and vehicles (C).

Figure 3-10: Samples and data collection procedure throughout the study *Source: Author's photos and design*

A. Participant Samples

Twenty participants were selected in each of the five locations (A, in Figure 3-10). All participants were males, non-smokers, healthy, and of the same ethnicity (Asian), nationality (Bengali), residence (Madinah city), and age category (25–35 years), and used the same accommodation and catering services (Appendix 7.4). A code was given to each participant according to his work area (Downtown, D001–D020, Quba, Q021–Q040, Awali, A041–A060, Sultana, S061–S080 and Al-Aziziyah, Z081–Z100). One participant from each of the five locations held the air-sampler device (a total of five individual air-sampler devices) during working hours for one day only per month, as shown in Figure 3-10. Two urine samples were collected, one in the early morning and another at the end of the 8-hour work shift, from the participant carrying the device on a monthly basis during the study year (Table 3-6). Therefore, samples were collected from twenty different participants every month in each of the five locations. Much more details about the data availability regarding locations, participants and samples are found in Table 3-7.

Table 3-6: Time plan for collecting air (individual and ambient) and urine samples (morning and after work)

Islamic Calendar	Gregorian calendar	Number of samples					
		Location 1	Location 2	Location 3	Location 4	Location 5	Total
Rabi al-awwal 3/1438	1 Nov–29 Dec 2016	20	20	20	20	20	100
Rabi ath-thani 4/1438	30 Dec 16–28 Jan 17	20	20	20	20	20	100
Jumada al-ula 5/1438	29 Jan–27 Feb 2017	20	20	20	20	20	100
Jumada al-akhirah 6/1438	28 Feb–28 Mar 2017	20	20	20	20	20	100
Rajab 7/1438	29 Mar-26 Apr 2017	20	20	20	20	20	100
Shaban 8/1438	27 Apr–26 May 2017	20	20	20	20	20	100
Ramaḍan 9/1438	27 May–24 Jun 2017	20	20	20	20	20	100
Shawwal 10/1438	25 Jun–23 Jul 2017	20	20	20	20	20	100
Dhu al-Qadah 11/1438	24 Jul–22 Aug 2017	20	20	20	20	20	100
Dhu al-Ḥijjah 12/1438	23 Aug–20 Sep 2017	20	20	20	20	20	100
Muḥarram 1/1439	21 Sep–20 Oct 2017	20	20	20	20	20	100
Ṣafar 2/1439	21 Oct–18 Nov 2017	20	20	20	20	20	100
Total		**240**	**240**	**240**	**240**	**240**	**1200**

B. Fixing Site Mentoring Stations

The sites of each air monitoring station were carefully selected to be as far from any gas stations or air pollution sources as possible. The high-volume air samplers were placed inside a frame related to the Madinah Municipality on the edge of a pavement in each of the five locations. CO concentration records were obtained from Awali air-mentoring station only due to the unavailability of such stations in any of the other locations (Downtown, Quba, Sultana and Al-Aziziyah).

C. Vehicle Counting and Classification

Madinah Municipality used a digital video system “Miovision Scout system (C)” in its streets, as is shown in Figure 3-10 to monitor traffic. Data from this system were then analysed both automatically and digitally to identify the numbers and types of motor vehicle. Small vehicles are motor vehicles with four wheels designed and constructed for the carriage of passengers and comprising no more than eight seats and having a maximum mass of 3.5 tonnes, e.g. personal cars and vans. Medium vehicles are motor vehicles with at least four wheels designed and constructed for the carriage of goods having a mass ranging between 3.5 and 12 tonnes such as ambulance. Large vehicles having a mass exceeding 10 tonnes including trailers and semi-trailers such as lorries. Data were then stored on a Secure Digital (SD) card and has a remote-control feature to allow for downloading data via the internet. The Scout system was easy to handle and can be operated by one person. The low-light camera unit was always mounted on the top of a lightweight column. Before extending the mast, we adjusted the angle of the camera to obtain a full view of all the passages and void direct alignment with sunrise or sunset. It has a 5.6-inch wide-angle camera and navigation system with maps and a timer, and operates in all weather conditions in temperatures from $^{-}$40–80°C. The system has a control box with a power source which can be filmed for a whole week, a memory of up to 64 GB, and a pole length of 7.6 metres. The video was then uploaded to Traffic Data Online 4.4, which analyses incoming data and produces statistical reports and figures that make it easier to read and benefit from its output. It is important to note that these data were obtained from Madinah Municipality and the only information available was numbers and types of vehicle (small, medium and large).

D. Participants' Route

Participants were instructed to walk not more than 0.2 miles around the fixed station (Figure 3-10) in order to monitor air pollution in their urine samples and ensure accurate and reliable results for analysis when compared to the station records. To ensure that participants adhered to this route during their working hours, Strava was downloaded onto their smartphones in order to show their route on the map, travel distance, duration, and other health features (Appendix 7-11).

3.7. Sample Analysis

The Madinah Regional Municipality offered unlimited use of its well-equipped laboratory Figure 3-11, air quality monitoring equipment, personnel, and other required data. The laboratory is accredited according to ISO/IEC 17025, and equipped with the most advanced and modern technical items, ensuring high quality analysis with the highest accuracy and precision by highly educated and qualified staff. Sample preparation and analysis was carried out as soon as the samples reached the laboratory.

Figure 3-11: Madinah Municipality's Laboratory *Source*: (Lab, 2017)

3.6.1 Air Sample Collection

Effective air sampling requires a quality pump with membrane filters and cassettes, size selective sampling heads, media holders, impingers, and sampling bags.

A. Individual Air Sampler

A low-flow individual air sampling pump device (Gilian LFS-113) was used. The Teflon filters (37 mm diameter) were mounted in a conductive polypropylene filter-holder (cassette). Participants carried a pump around their waists connected via a silicon tube to the individual air sampling device, which was attached to the clothing on the right shoulder ≃10cm away from their mouth, in order to be in their breathing zone (Figure 3-7). All participants were subjected to intensive training on how to operate and handle these devices correctly.

Particles ($PM_{2.5}$) that contained BaP were collected on the Teflon filter made from polytetrafluoroethylene (PTFE) (Zefluor™, 37 mm diameter, 2.0 µm pore size; SKC Inc.). This provided a very low background in the analysis and an efficient retention of agglomerated particles and BaP bound to carbon due to the matrix of randomly oriented fibres, which are bonded together in several layers (depth filter) and retain particles on the surface and throughout the matrix (ASTM, 1999). Individual air samplers collected air samples during the working day (eight hours), with a flow rate of 2.0 L/min of (200–1000 L/ sample). Immediately after sampling, the filter was transferred carefully with forceps to a scintillation vial. The scintillation vial was capped and wrapped in aluminium foil to avoid loss of analytes due to sublimation and degradation by light. All samples were stored at $^{-}20$°C while waiting for analysis.

1. Air Sampling Device Preparation

- Filter cassettes were assembled prior to starting field work
- The two halves of the cassette were firmly and completely seated against each other to prevent sample material from bypassing the filter. A good seal between the filter and the cassette halves was ensured
- Sampling pump batteries were checked to ensure they were fully charged
- Individual sampling pumps were calibrated before and after each day of sampling

- Sampling pumps at the temperature and atmospheric pressure were calibrated at the location where samples were collected;
- Pre-sampling calibration data (such as pump serial number and flow rate), temperature and atmospheric pressure of the calibration location were recorded;
- Each sample was labelled with a unique sample code.

2. Sample Preparation —Extraction, Cleaning and Concentration

The air samples collected on Teflon filters were extracted according to NIOSH methods 5515 (NIOSH, 2010).

3. Extraction

Cyclohexane is recommended for extraction of BaP from vehicles and diesel exhaust particulate (Breuer, 1984).

a. 5.0 ml of Cyclohexane was added to each scintillation vial containing a filter. Start media and reagent blanks at this step were then capped and left to stand for 15–20 min in an ultrasonic bath;

b. Soxhlet extraction was performed in case there were large amounts of highly adsorptive particulate matter.

4. Calibration, Quality Control and Calculations

a. Calibration was performed daily according to at least six working standards (10, 5, 1, 0.2, 0.1 and 0.05 ng/ml). Aliquots of calibration stock solution from BaP were diluted with toluene in 10 ml volumetric flasks;
b. Intersperse working standards and samples in the measurements;
c. Calibration graphs were prepared (peak area vs. µg of BaP per sample).

5. Recovery and Desorption Efficiency

a. Recovery (R) from filters and desorption efficiency (DE) from sorbent tubes were determined at least once for each batch of filters and sorbent tubes were used in the range of interest;

b. Using a microliter syringe or micropipette, four filters at each of five concentration levels were fortified with calibration stock solution (5, 1, 0.2, 0.1 and 0.05 ng/ml). The filters were left to dry overnight in the dark then analysed as mentioned before. Recovery charts (R vs. amounts found) were plotted.

B. High-Volume Air Sampler

The PUF high-volume air sampler (GPS11-BL, Thermo Scientific, USA) was used. The aerodynamic design of these high-volume samplers made them capable of continuously collecting indoor or outdoor $PM_{2.5}$ for 24 hours using Mass Flow Control (MFC). They had a high-speed motor, durable all-weather shelter and rugged electronic components for accurate sampling. They were also supported by a protective shelter that ensures the horizontal position of the filter surface. They were designed to meet international standards and U.S. EPA reference standards for high volume air sampling of $PM_{2.5}$ particulates (Method TO-13A), as well as the determination of BaP in ambient air using a gas chromatograph (GC).

1. Preparation of PUF Cartridge

Before sampling, the filters were preheated at 400 °C for 48 h in a furnace, wrapped in clean foil, placed in a cardboard box, and sealed in an airtight metallic container. The glass cartridge PUF was cleaned before use by immersing it in 100 ml of dichloromethane, put in ultrasonic ting at 20 °C for 30 min, and then dried in a sealed metal container under a stream of nitrogen. Finally, the PUF glass cartridge was sealed in an airtight plastic bag and stored in the freezer at $^{-}18$°C.

2. Sample Collection

Daily air samples were collected simultaneously from the five locations during the study year. The PUF high volume air sampler was located at the Municipality's fixed-site air sample station on a pavement (Figure 3-10). The sampler was operated for 24 h to obtain the average daily levels of airborne BaP.

Volumes of air in the range of 240–300 m^3 over a 24 h period were drawn by the sampler through a quartz micro fibre filter (TE-QMA4 10.16 cm) to collect BaP in the $PM_{2.5}$ particulate matter, followed by an absorbent PUF to collect compounds present in the gaseous phase. Samples were kept in an aluminium shipping container (containing the filter and PUF sorbents), chilled at <4 °C, and sent to the laboratory for analysis.

3. Sample Preparation —Extraction, Cleaning and Concentration

Samples were analysed for BaP using the modified method of (Delgado-Saborit *et al.*, 2013):

a. The sample filter and PUF cartridge were spiked with 1000 pg/µl deuterated internal standards for quantification;
b. Filters were immersed in 100 ml of dichloromethane and then ultrasonicated for 15 min at 20°C;
c. The extract was subsequently dried using nitrogen steam and then cleaned using a chromatography column filled with 2 grams of 1.2% deactivated Florisil with 0.5 g of anhydrous sodium sulphate;
d. The extract was then concentrated to 50 µl under a gentle nitrogen flow;
e. The same steps (b–c) were carried out for the PUF cartridge then concentrated to 10 ml using nitrogen steam;
f. Finally, the concentrates of filter and PUF sorbant extracts were injected to GC-MS for the determination of BaP concentration.

4. Detection and Determination by Gas Chromatography with Mass Spectrometry

The detection of BaP in the extracts of the individual air and high-volume air samplers was accomplished by an electron ionisation gas chromatograph/mass spectrometer (EI-GC/MS) (6890, Agilent Technologies) equipped with a Tandem Mass Spectrometer (5973N, Agilent Technologies). The GC was equipped with a temperature-controlled, non-polar capillary column (Agilent HP-5MS, 30m, 0.25 mm ID, 0.25 µm film thickness – 5 % phenyl polysiloxane) interfaced directly to the MS ion source. Helium was used as a carrier gas for analyte separation at a flow rate of 1.0 ml/min.

The GC/MS was tuned before work using a 50 ng/µl solution of decafluoro triphenyl phosphine (DFTPP) and the operating conditions were set up as follows:

A splitless injection of 2µl, injection temperature of 280 ºC and the oven temperature programme was 80 ºC, ramped at 15 ºC/min to 185 ºC (held for 5 min), ramped at 10 ºC/min to 240ºC (held for 10 min), and ramped at 3 ºC/min to 276 ºC (held for 5.5 min). The mass spectrometer used the electron impact mode of 70 eV. The transfer line temperature was 290 ºC, and solvent delay was set to 4.2 min. The detector was set to quantify the BaP and other BaP analytes using monitoring (SIM) mode covering specific masses ranging from 150–350 atomic mass units with a dwell time of 50 to 100 milliseconds per ion using MSDS Chemstation software.

5. Calibration of GC/MS and Quality Control

a. Stock Standard of BaP

A stock standard solution of BaP was prepared with a concentration of 2.0 µg/µl (weight 0.2g from standard material in 100 ml dichloromethane) and stored at 4 ºC protected from light.

b. Calibration Curves of BaP

A series of calibration solutions of BaP were prepared in dichloromethane and a six-point calibration curve (0.0, 0.1, 0.25, 0.50, 1.25 and 2.50 ng/µl) was plotted.

c. Sample Analysis by GC/MS

Each sample extract was analysed by GC/MS and quantitated using the internal and external standard. The GC/MS was tuned and calibrated before injection.

All sample extracts were left to take ambient temperature (≃1 hour) before injection. All samples were injected under the same instrumental conditions (method, calibration, temperature program). Final quantitation of BaP and calculation of concentration was conducted as in:

$$C = \frac{A_x\, I_s\, V_t\, D_f}{A_{is}\, V_i\, (RRF)}, \quad (\frac{ng}{m^2})$$

where,

C = The concentration

A_x = The area response for the compound to be measured, counts

I_s = Amount of internal standard, ng/µl

V_t = Volume of final extract, µl

D_f = Dilution factor for the extract

A_{is} = Area response for the internal standard, counts

V_i = Volume of air sampled, m3

RRF = Mean from the most recent initial calibration, dimensionless

3.6.2 Urine Sample Collection and Analysis

1. Sampling and Storage

Two urine samples (morning and after-work sample) were collected from 20 different janitors in the five locations during the study year, as shown in Table **3-6**. All urine samples were collected in sterilised polypropylene specimen containers without preservative and transported in coolers. The samples were stored protected from light in 500 ml amber glass containers and stored frozen at $^{-}$20 °C to prevent decomposition of 1-hydroxy metabolites of BaP prior to preparation and analysis.

For sample analysis, approximately 50 ml of a sample was sufficient for a triplicate analysis. The samples can be kept for at least one year without loss of 1-OHP (Jongeneelen, 2001).

2. Urine Sample Preparation

Urine samples were analysed for 1-OHP by HPLC using a modification of the methods described by Jongeneelen (2001) and Elovaara *et al.* (2006), as below:

a. The frozen samples were slowly thawed and shaken before analysis;

b. A 10 ml aliquot of urine sample was adjusted to a pH=5 with 10ml 1.0 M acetate buffer;

c. A 25 μl of β-glucuronidase/arylsulfatase was added to the sample and vortexed for 10 seconds. This mixture was incubated overnight (16 hr) at 37 °C in an electronically controlled rotary shaking bath for enzymatic hydrolysis;

d. A sample purification cartridge, packed with C18 reversed-phase liquid chromatographic material (Sep-Pak C18 cartridge, Waters) was used for sample extraction. The cartridge was first primed with 5 ml of methanol followed by 10 ml distilled water. The treated urine was filtered, passed through the cartridge at a flow rate of 1 ml/min. and then washed with 3 ml distilled water and 3 ml of 50% methanol in water, and 8 ml methanol was added as final elution;

e. The solvent was evaporated at 40 °C using a constant flow of nitrogen (150 ml/min). The residue was dissolved in 2 ml of methanol, transferred to amber HPLC auto sampler vials and stored at $^{-}$20 °C until determination.

3. Detection and Determination by HPLC

The detection of 1-OHP was performed using an HPLC (1100 Series; Agilent, Santa Clara, CA, USA, with a fluorescence detector (242 nm excitation wavelength and 388 nm emission wavelength) and a Zorbax C18 column (5 mm, 4.6 mm diameter, 150 mm length; Agilent). The HPLC analysis was performed in 25 min using a mixture of methanol and water (71:29 % by volume) as the isocratic mobile phase.

4. Calibration of HPLC and Quality Control

A calibration curve was constructed with the pooled urine using the method of standard addition to minimise matrix interference. An intermediate standard was serially diluted in the pooled urine to prepare nominal 1-OHP concentrations of 0, 1, 5, 10, 15 and 20 ng/ml. The resulting data produced a linear standard curve with an intercept close to 0.0. The instrumental limit of detection was ~0.50 ng/ml. Extraction recovery of urinary 1-OHP was 84.1 % at 2.5 ng/ml of urine. All urine samples were analysed and corrected for creatinine with a final unit (μmol/mol creatinine) to overcome such factors as urine output or dilution which might influence the concentration of 1-OHP (Hinwood *et al.*, 2002). Urinary creatinine was determined according to the Jaffee method (Taussky and Kurzmann, 1954), depending on direct reaction of urinary creatinine with alkaline picrate resulting in the formation of a red colour, with the intensity measured at 505 nm using a UV/Vis spectrophotometer.

3.8. Meteorological Data

The General Authority of Meteorology and Environmental Protection in Madinah provides the author with the meteorological data including temperature, relative humidity, wind speed and direction. They did not provide any other details about the location of the meteorological stations except that the provided data were the average of 20 meteorological stations distributed all over Madinah city in sites different from the five locations of the study and they could not provide even a map to specify the points of those meteorological stations. However, there are at least 3 stations from 20 stations which are closer to the 3 polluted locations of this study (Downtown, Quba and Awali).

3.9. Statistical Analysis

Data were entered using MS-Excel and analysed using SPSS version 18 and Minitab version 18. Figures were drawn using Minitab, Excel and R-software.

The study included two types of data:

- Numerical continuous data representing measurements such as ambient and individual BaP concentrations, CO concentration, urinary 1-OHP concentration, and weather factors such as temperature, relative humidity and wind speed;
- Numerical discrete data that can be counted but not measured, such as traffic counts;
- Categorical data describing characteristics such as locations (five), holy and normal months (12), traffic density (traffic and control), and holy events (Ramadan, Hajj and normal months).

Medians, interquartile range, minimum, maximum and skewness statistics were computed. Box plots, time series plots and calendar plots were plotted for different variables such as ambient, individual BaP, CO levels and traffic volume. These plots were also drawn to compare traffic and control locations.

3.9.1. Test of Normality

All data were examined for normality using a Shapiro-Wilk test and skewness calculation using Pearson's coefficient of skewness. The alpha level of significance in the present study was considered at p.<0.05. The Pearson's coefficient of skewness K_2, with the median given by:

$$K_2 = \frac{3(\overline{X} - M)}{S},$$

where, $\overline{X}$ is the mean, M the median, and S the standard deviation. Results were considered positively skewed if the ratio of the skewness value to its standard error was > 0.2, and negatively skewed if the ratio was $< {}^{-}0.2$. Data were interpreted according to Bulmer (1979), as follows:

- Normally distributed skewness was ≈0 [${}^{-}0.5$ to 0.5];
- Negative skewness [${}^{-}1$ to ${}^{-}0.5$]: in this range, skewness was considered moderate while for values less than or greater than ${}^{-}1$, skewness was considered high;
- Positive skewness [0.5–1]: in this range, skewness was considered moderate.

Most variables in the present study were found to be not normally distributed, even after transformations (power, exponential, square root, square, cubic and inverse).

3.9.2. Non-Parametric Tests

As a result of the non-normal distribution of data, non-parametric Kruskal-Wallis test was used to compare the median ranks of the variables between different groups. If the Kruskal-Wallis was significant ($p<0.05$), individual Mann Whitney tests were carried out according to the number of combinations, with the adjustment of the p-value using Bonferroni correction. Individual Mann Whitney tests were considered significant at a Bonferroni-corrected p-value <0.01 for pairs of locations and Bonferroni-corrected p-value <0.004 for pairs of months.

3.9.3. Correlation, Scatter and Curve Estimation Regression Analysis

The bivariate Spearman rank correlation was used for non-parametric data to measure the strength of association between two variables and the direction of the relationship. Correlation was considered significant at $p < 0.05$. The following formula was used to calculate Spearman rank correlation:

$$\boldsymbol{\rho = 1 - \frac{6 \sum {d_i}^2}{n(n^2-1)}},$$

where $\boldsymbol{\rho}$ is the spearman rank correlation, $\boldsymbol{d_i}$ denotes the difference between the ranks of corresponding variables, and $\boldsymbol{n}$ denotes the number of observations.

The scatterplots with fit lines were created to explore the scatter of values and confirm the existence of a relation.

3.9.4. Curve Estimation Regression Analysis

Non-parametric curve estimation is most appropriate when the relationship between the dependent and the independent variables is not linear. In this study, ambient and individual BaP results were obtained using kernel regression (Herrmann, 1997; Heidenreich *et al.*, 2013) in which the best smoothing lines adapt to the original data. The smoothing method was applied for the time series plots to adapt the gap in data collections during weekends.

Data were screened graphically using scatter plots to explore the type of relation between independent and dependent variables (linearly, power, inverse, logarithmic etc.). With the linear variables, a simple linear regression model was used. For variables not linearly related, different transformations of data were examined to find the best fit, and so the fit line was used for linear relations and curve fits for power, square, or inverse relations between variables. The power curve showed the best fit as it had best R-square and the least square root of the variance of the residuals. Residual values (the difference between the observed data point and the predicted point from the regression equation) were used to assess and improve the curve. A separate curve fit was produced for each dependent variable. A strong correlation between the predicted and actual results produces a good and accurate regression plot.

3.9.5. Time Series Graphs, Box plots and Calendar Plots

A time series graph is a line graph of repeated measurements taken over regular time intervals. In this study, time series graphs were drawn to show the trend of the study variables over time for long-term trends, and seasonal or cyclical variations. Microsoft Excel 2016 was used to make time series graphs. The calendar plot corresponds to the time series chart but makes it easier to spot the approximate concentrations over the course of a long-time span, such as months or years, and compare them. In this study, the calendar plot illustrates how the study variables alter with days of the week, either workdays or the weekend, throughout the study year. R software (Version 1.1.463, ©2009–2018 RStudio, Inc.) was used to make the calendar plots by using 'openair' package version 2.6-1. The boxplots were plotted using Minitab 18 (©2017 Minitab, Inc.) to demonstrate the statistical descriptive analysis in terms median, minimum, maximum, IQR, skewness and outliers.

3.9.6. Auto-Correlation

Autocorrelation is a statistical method used for time series analysis which represents the degree of similarity between a given time series and its lagged version over successive time intervals. It is very important to uncover hidden patterns in the obtained data. Specifically, it is used to help in identifying seasonality and trend in the time series data. Additionally, analysing the autocorrelation and partial autocorrelation functions is necessary for selecting the appropriate ARIMA model for the time series prediction.

In spite of its importance; auto-correlation was not applied to the time series data in this study due to the lack of data during holidays and weekends which represents about 32.5% of the data due to 116 off-days. This missing data was accounted for by measuring 1-OHP emerging in the urine of the participants as a cumulative result after three continuous working days as evidence of exposure to BaP pollution during the weekends.

3.10. Data Availability

The general background about the study and the data obtained are summarised in Table 3-7, representing the scheme used to achieve the objectives of the study during one Islamic calendar year (354 days) from 1/4/1438 to 30/3/1439 —corresponding to the Christian calendar as 30 December, 2016 to 18 December, 2017.

A total of 1,200 air samples were collected throughout the study period, excluding the weekends (Fridays and Saturdays) from individual air samplers carried by targeted city janitors in the five locations specified for the study (Sultana and Al-Aziziyah as control and Downtown, Quba and Awali as traffic locations). Another 1,200 air samples were collected from the filters of high-volume air samplers placed at the same locations, resulting in 2,400 records of BaP as a PAH-air pollutant marker. Additionally, 1,200 urine samples were collected from the targeted city janitors in the early morning before working hours, and another 1,200 urine samples after work, resulting in 2,400 records of urinary 1-OHP as another PAH-air pollutant marker.

Supporting these results, air quality was recorded 24 h for CO rates during the study period.

Table 3-7: General Background of Study Data

Study period (Islamic months)		**Study locations**		**Daily records**		**No. of participants**	**Type of samples**					
							Air samples			**Urine samples**		
		Control locations	**Traffic locations**	**Traffic records**	**Metrological records**		**Source of samples**	**No. of samples collected**	**Marker**	**Source of samples collected**	**No. of samples collected**	**Marker**
M4	Rabi ath-thani	2 locations	3 locations	4248 records *(8h/354 days)*	4248 records *(354 days)*	100 *(20 persons x 5 locations)*	Individual Air Sampler *	**1,200** *(240 days x 5 locations)*				
M5	Jumada al-ula	Sultana Alaziziah	Downtown Quba Awali	Cars Medium vehicles Heavy vehicles	Temperature Relative Humidity Wind Speed Precipitation							
M6	Jumada al-akhirah						High-Volume Air Sampler *	**1,200** *(240 days x 5 locations)*	BaP	Morning urine	**1,200** *(240 days x 5* locations)	
M7	Rajab											
M8	Shaban											
M9	Ramadan						**Total**	**2,400**				
M10	Shawwal											1-OHP
M11	Dhu al-Qadah											
M12	Dhu al-Ḥijjah						Air quality records**	**4,248** *(24 h x 354 days)*	CO	After work urine	**1,200** *(240 days x 5 locations)*	
M1	Muharram											
M2	Ṣafar											
M3	Rabi al-awwal									**Total**	**2,400**	

** missing records during weekends (Fridays and Saturdays = 114 days)*
*** Air quality records from one location only (Awali)*

CHAPTER FOUR

BaP Measurements in Madinah over One Year and the Most Influential Factors on Concentrations

Chapter 4: BaP Measurements in Madinah over One Year and the Most Influential Factors on Concentrations

4.1.Introduction

This research had four objectives, as previously mentioned in the introduction. This chapter addresses objectives one and two. The first objective covers the determination of BaP concentrations and hot spots in Madinah compared to the background sites (control location), with the second objective involving the identification of major traffic (traffic volume and ambient CO) and meteorological (temperature, relative humidity, precipitation) variables within the normal months and religious events that could affect air pollution with BaP during one Islamic calendar year in Madinah city.

The chapter begins by presenting the results and analysis of traffic volume and CO levels in different months and events, followed by the results and data analysis of meteorological measurements. Finally, the results and data analysis are presented of ambient and individual BaP in Madinah city at the five studied locations (Downtown, Quba, Awali, Sultana and Al-Azizyah) during one Islamic calendar year. At the end of the chapter, the correlation between parameters is represented, the interpretation of the findings in the light of previous studies is discussed, and then conclusions are drawn and summarised.

4.2.The Relationship between Traffic Volume and Air Pollution

Traffic volume deals mainly with the number of vehicles moving on roads at a particular location and time, and has a direct and proportional effect on environmental pollution. This section tries to correlate the volume of traffic and associated air pollution by representing the findings of the traffic volume in Madinah at the five studied locations during the study year. The traffic count was undertaken automatically by recording the number of vehicles crossing a road in both directions every day during the study period. Generally, traffic is expected to be high during the morning and evening and relatively low at other times. This is because from 3pm to around 11pm (evening) people are either travelling to or from their places of worship to mark these important religious events. Morning traffic is expected to be high because this is the time people are travelling to their work places. Table 4-8 shows the median, minimum and maximum number of vehicles per day passing the three traffic locations (Awali, Quba, and Downtown) and two control locations (Sultana and Al-Aziziyah) during the study year, as well as the interquartile range and skewness.

The descriptive data shows that the highest median traffic volume was recorded by Awali and the lowest by Al-Aziziyah, with Awali recording 23,354 vehicles/day, Quba 21,079 vehicles/day, Downtown 17,468 vehicles/day, Sultana 11,805 vehicles/day, and Al-Aziziyah 4,178 vehicles/day.

The statistical analysis of the traffic volume data using the Kruskal-Wallis Test with H-value = 1,430.5 ($p.<0.001$) indicates that there was a marked difference between the distributions of traffic volume in the five locations. The Mann Whitney Test was carried out ten times (five locations and two combinations) with Bonferroni correction ($p.< 0.01$), and the marked differences in traffic volume between traffic and control locations were denoted by letters (a, b, c and d) as shown in Table 4-8. This indicates that the distributions of traffic volume in traffic locations (a and b) were markedly different, both from each other and the control locations. In addition, the traffic volume distribution in the control locations (c and d) were statistically different from each other.

In this response, the statistical analysis confirms the conclusion drawn from the descriptive data. Moreover, the skewness was calculated using Spearman's median coefficient to check the distribution of data showing that all locations have right-tailed distribution with moderate/high positive skewness, confirming the non-normality of data and that the mean of the traffic volume was greater than its median. The right-tailed distribution (skewness) of the traffic volume data was because of relatively high traffic volumes during the three and a half holy months compared to other normal months, as will be specified in detail further on.

Table 4-8: Traffic volume (vehicles/day), at five locations during the study year

Location	Median	Minimum	Maximum	IQR	Skewness	Distribution symmetry
Downtown[a]	17,468	14,385	33,942	10,179	0.96	+
Quba[a]	21,079	16,984	34,436	8,912	0.89	+
Awali[b]	23,354	18,104	34,512	7,641	0.80	+
Sultana[c]	11,805	8,813	16,630	2,097	0.53	+
Al Aziziyah[d]	4,178	2,837	10,419	894	1.90	++

Locations with different letters (a, b, c, d) were markedly different in their data distribution, IQR: Inter Quartile Range, +: moderate positive skewness (>0.5 and ≤1.0); ++: high positive skewness (>1.0), SES: Standard Error of Skewness = 0.130)

For further interpretation, the box plots demonstrated in Figure 4-1 showed a marked rise in traffic volume at the traffic locations (Downtown, Quba and Awali). The maximum counts in the three traffic locations reached 34,512 vehicles/day while the minimum was 14,385 vehicle/day in both directions. There were also marked variations in traffic volumes in the upper quartiles of the three box plots, representing the three traffic locations. The traffic volume in traffic locations Awali, Quba and Downtown were markedly higher compared to Sultana, and consequently they were also noticeably higher than Al-Aziziyah, which recorded the lowest traffic volume. The control locations (Sultana and Al-Aziziyah) expressed short whiskers and narrow lower quartiles (Q1) in the box plots, indicating low variation in traffic volume, especially in Al-Aziziyah. The wide variation in traffic volume in the traffic locations may be attributed to high traffic volume during the holy months and low traffic volume during normal months. The low traffic volume variation in the control locations may be due to relatively constant traffic volume in these locations throughout the year.

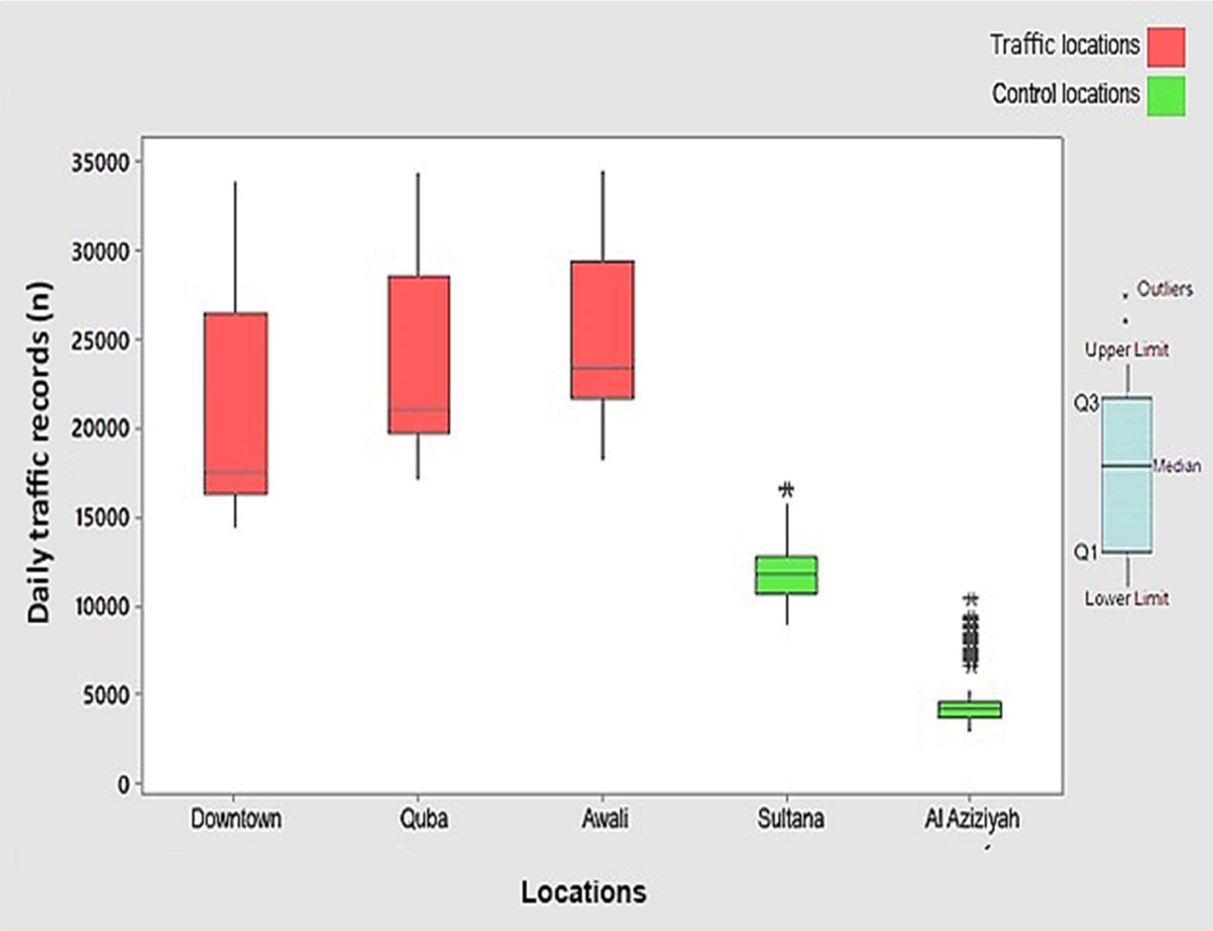


Figure 4-11: Box plot of traffic volume (vehicles/day) at five locations during the study year

To demonstrate these previously obtained results further, a time series plot illustrating the number of vehicles/days in the five locations during the study year is shown in Figure 4-12. The three curves of the traffic locations were similar and both of the control locations also appeared to be similar to each other. The influence of holy events was noticeable in Sultana, with the higher number of vehicles (slightly above 15,000 vehicles/day) compared to Al-Aziziyah, which was almost unaffected as the maximum number of vehicles was just below 5,000 vehicles/day. On the other hand, traffic locations Awali, Quba and Downtown reached the maximum counts of vehicles/day at just below 35,000.

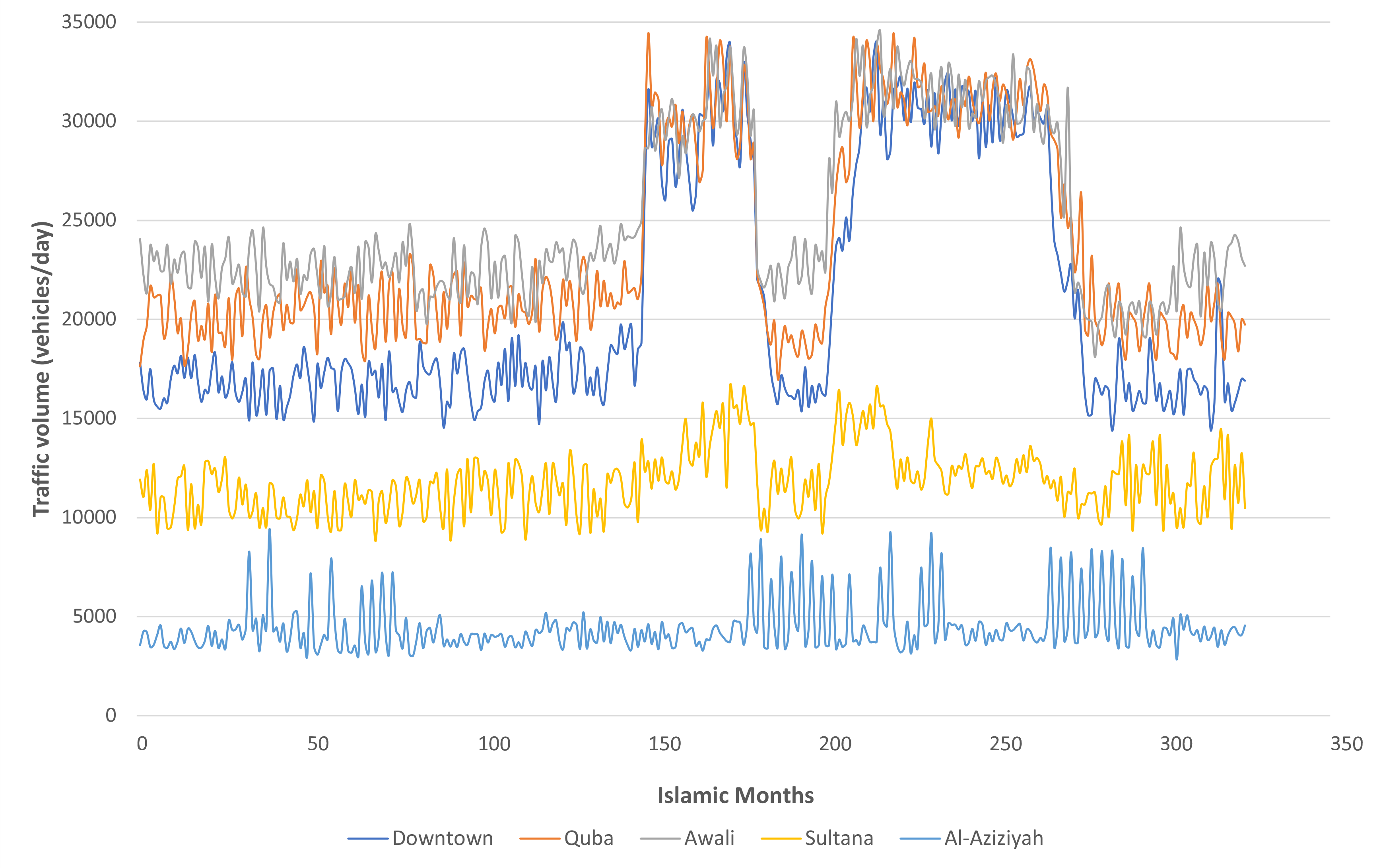


Figure 4-12: Time series plot of traffic volume (vehicles/day) at five locations during the study year

4.2.1. Traffic Volume per Month during the Study Year

Table 4-9 shows the descriptive statistics of traffic volume throughout the months of the study year in traffic locations at Madinah across 354 records per year, contributing to a total of 1,062 records in the three traffic locations (Awali, Downtown and Quba); there were no missing data.

After applying the Kruskal-Wallis Test with H-value = 579.4 ($p.<0.001$), it was concluded that there was a marked difference between the distributions of traffic volume in the traffic locations, and 66 Mann Whitney Tests (12 months with two combinations) were applied with a Bonferroni p-value of < 0.004, contributing to a statistically significant difference of traffic volume throughout the months of the study year carrying different letters (a, b, c). Medians were higher in all holy months (M9, M11, M12 and M1) compared to normal ones.

The results of the Kruskal-Wallis and Mann Whitney tests showed that, during the Islamic year in the traffic locations, the distribution of traffic volume in the holy months was different from that of the normal months (denoted by c), and also different from the distribution of traffic volume in M1 (denoted by b). These variations may be because during the holy months the number of people travelling along the highways of Madinah city to perform religious rites, especially in areas located in traffic locations, increases significantly compared to other months. The difference of traffic volume in M1 was brought about by the fact that the first half of that month was holy, which is associated with high traffic, while the second half of the month was normal and hence associated with normal traffic volume.

Table 4-9: Traffic volume (vehicles/day) at traffic locations during the study year

Month Number	Month Name	Median	Minimum	Maximum	IQR	Skewness	Distribution symmetry
M4	Rabi ath-thani[c]	19,754	18,913	21,016	833	-0.030	≈0
M5	Jumada al-ula[c]	19,645	18,153	21,110	885	-0.266	≈0
M6	Jumada al-akhirah[c]	19,987	18,521	21,639	1,139	-0.122	≈0
M7	Rajab[c]	19,802	18,877	21,706	850	-0.317	≈0
M8	Shaban[c]	20,753	19,587	22,035	982	-0.331	≈0
M9*	Ramaḍan[a]	29,789	27,965	33,827	2,811	0.234	≈0
M10	Shawwal[c]	19,758	18,505	33,137	4,084	0.776	+
M11*	Dhu al-Qadah[a]	31,379	27,189	33,824	1,315	1.10	++
M12*	Dhu al-Ḥijjah[a]	30,946	29,894	32,450	750	-0.047	≈0
M1*	Muḥarram[b]	19,335	17,707	30,925	6,061	0.724	+
M2	Ṣafar[c]	19,566	17,707	21,548	1,301	0.002	≈0
M3	Rabi al-awwal[c]	19,573	18,348	21,548	696	-0.113	≈0

*Months with different letters (a, b, c) were markedly different in their data distribution, IQR: Inter Quartile Range, ≈0: approximately symmetric (≤0.5); +: moderate positive skewness (>0.5 and ≤1.0); ++: high positive skewness (>1.0), Standard Error of Skewness (SES) = 0.25; *: holy month*

For data analysis purposes, box plots were used, as shown in Figure 4-13, to demonstrate the shape of the daily traffic volume data distribution with its median value and variability. Holy months M9, M11 and M12 showed distinctly higher medians than the rest of the months. The short quartiles in all months (except M10 and M1) indicated mild variation (nearly stable) between the vehicle counts during these months. On the contrary, M10 and M1 showed wide variation in traffic volume demonstrated by their long upper quartiles (Q3) compared to their lower quartiles (Q1). The minimum traffic volume occurred in M10, and M1 was similar to that of the normal months, confirming that these two months are considered to be transition periods between holy months and normal months. However, the traffic volume returned to its basic level a few days after the beginning of M1, with the departure of all Ramadan visitors mostly within days of Eid Al-Fetr.

The wider variation in traffic volume in M1 was because the first half of the month was holy, and hence was associated with religious activities, resulting in high traffic volume. The second half of the month was associated with normal activities and can be considered a normal month, resulting in low traffic volume.

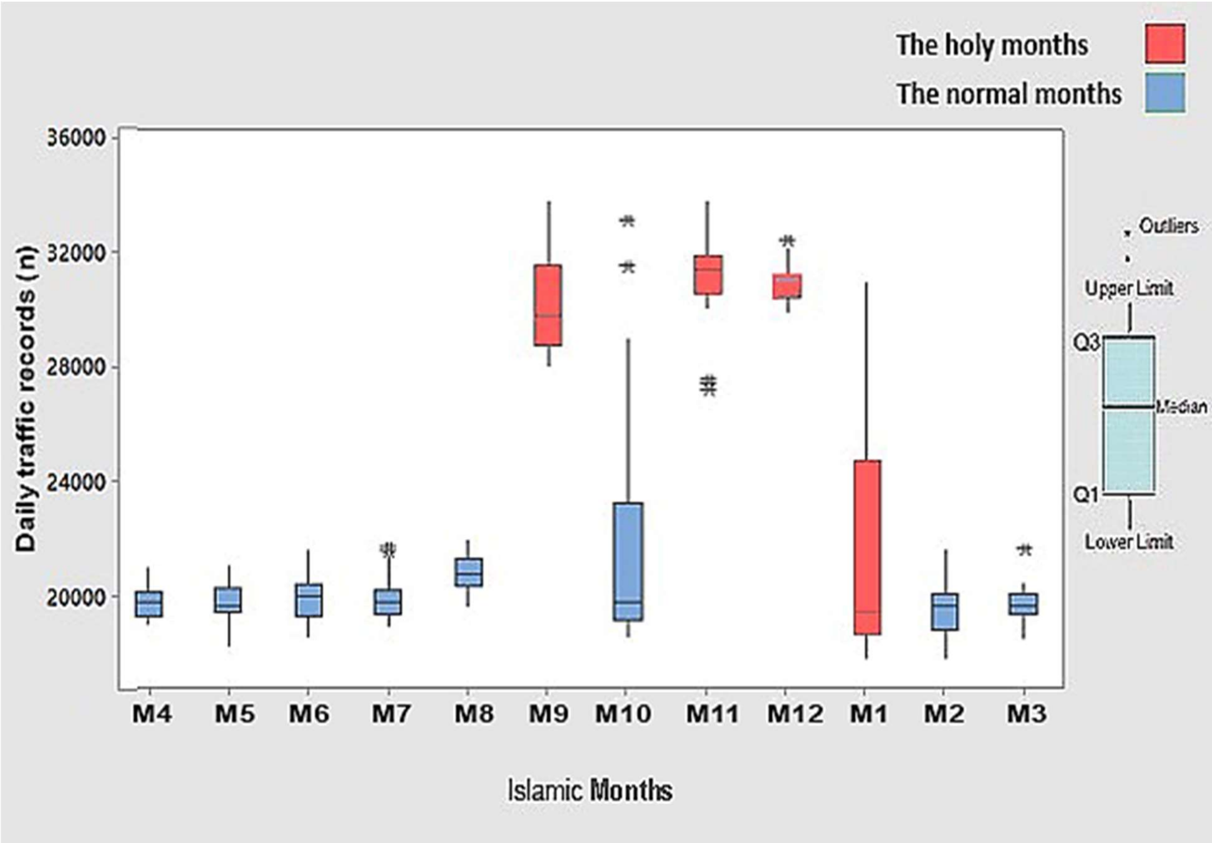


Figure 4-13: Box plot of daily traffic volume (vehicles/day) per month in traffic locations during the study year

In addition, the calendar plot in Figure 4-14 was used to make it easier to spot and compare the maximum daily traffic volume for each month in traffic locations in Madinah during the study year. The daily traffic volume was represented as a colour gradient, with the darker colour pointing to a heavier traffic volume on a specific day. The dark-coloured spots (hotspots) were recorded during the holy months M9, M11 and M12 as well as on the first four days of M10 and M1, considered transition months. The darkest colour gradient of more than 32,000 confirmed the maximum traffic volume in Awali, Quba and Downtown (34,512 vehicles/day, 34,436 vehicles/day and 33,942 vehicles/day, respectively), while the lighter colour gradient indicates fewer than 18,000 vehicles/day, confirming with the maximum vehicle count in the control locations of 16,630 vehicles/day in Sultana and 10,419 vehicles/day in Al-Aziziyah, as shown in Table 4-9.

It can be observed that the darkest colour gradient was in the holy months and the lightest gradient in the normal months. Transition months had a mix of dark and light gradients, confirming conclusions from the previous sections that the traffic volume varied between holy to normal months in the traffic locations due to the religious seasons having high population capacity and high transportation rates.

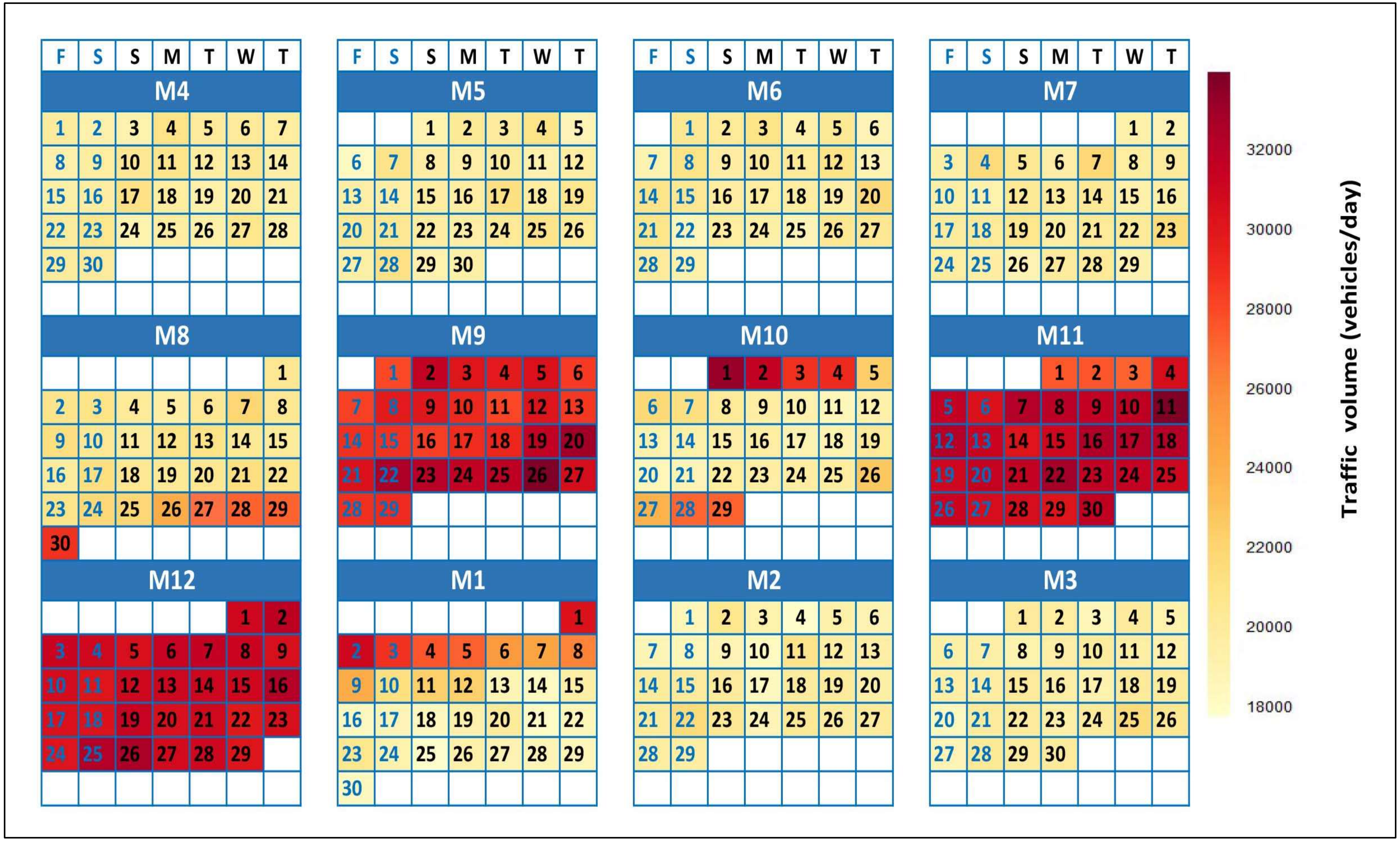


Figure 4-14 : Calendar plot of daily traffic volume (n/day) in traffic locations during the study year

For more clarification of the previous conclusions, the time series in Figure 4-15 was plotted to illustrate the types and number of vehicles/day in the traffic locations. The maximum daily numbers of medium and heavy vehicles as well as cars were observed during the holy months M9, M11, M12, and the beginning of M1, taking into consideration that the number of cars (>25,000) was much higher than the number of medium (<1,000) or heavy vehicles (>7,000). From this figure, it can easily be noticed that the quantity of all types of vehicles increased considerably during the holy months (M9, M11 and M12) and started to decrease with the departure of pilgrims and visitors and returned back to normal counts by the end of M1.

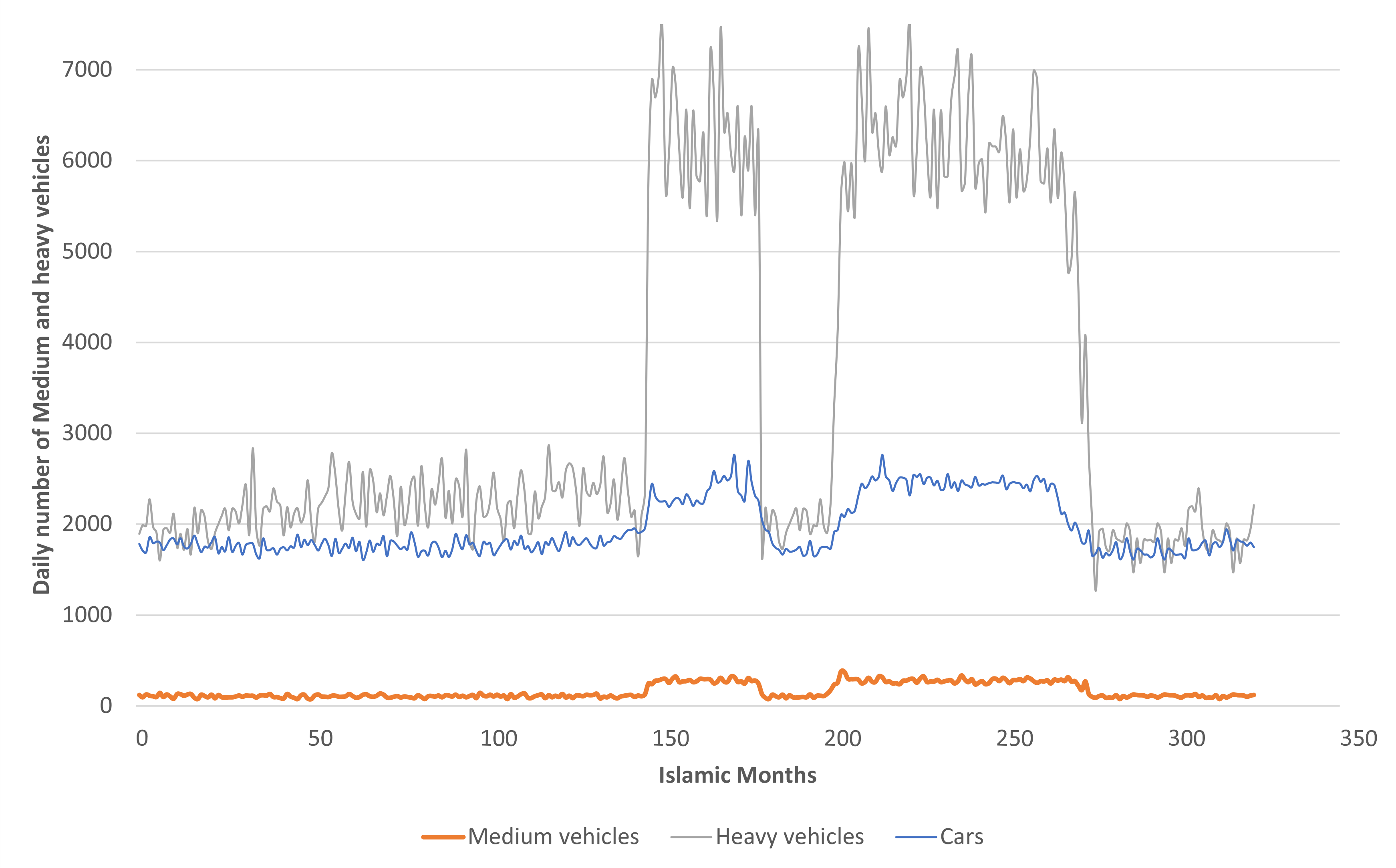


Figure 4-15: Time series plot for the type of traffic volume (vehicles/day) at traffic locations during the study year

4.2.2. Traffic Volume Data in Control Locations

The total number of traffic records per year was 354, in each of the two control locations, resulting in 708 records for the two control locations with no missing data. There was no significant difference between the distribution of data in the control locations throughout the months of the study year, as concluded from the Kruskal-Wallis test with H-value = 18.1 (p = 0.08) and illustrated in Table 4-10 and box plot Figure 4-13. The maximum traffic volume was 12,221 vehicles/day and the minimum was 6,160 vehicles/day, leading to the conclusion that control locations were far away from the traffic congestion and unaffected by the holy events during the study year.

Table 4-10: Traffic volume (vehicles/day) at control locations during the study year

Month Number	Month Name	Median	Minimum	Maximum	IQR	Skewness	Distribution symmetry
M4	Rabi ath-thani[a]	7,696	6,447	8,698	1,220	0.129	≈0
M5	Jumada al-ula[a]	7,888	6,270	10,792	1,583	-0.413	≈0
M6	Jumada al-akhirah[a]	7,966	6,034	10,206	1,920	-0.301	≈0
M7	Rajab[a]	7,599	6,176	8,547	1,065	0.164	≈0
M8	Shaban[a]	7,788	6,313	9,106	1,393	0.137	≈0
M9*	Ramaḍan[a]	8,663	7,444	10,203	1,625	0.144	≈0
M10	Shawwal[a]	8,144	6,350	11,423	2,597	0.125	≈0
M11*	Dhu al-Qadah[a]	9,418	7,496	12,221	1,775	-0.089	≈0
M12*	Dhu al-Ḥijjah[a]	8,355	7,570	8,946	550.3	0.018	≈0
M1*	Muḥarram[a]	8,072	6,857	10,874	1,645	-0.220	≈0
M2	Ṣafar[a]	8,046	6,160	9,369	1,499	0.212	≈0
M3	Rabi al-awwal[a]	7,947	6,636	9,369	1,362	0.198	≈0

*IQR: Inter Quartile Range, ≈0: approximately symmetric (≤0.5), SES: Standard Error of Skewness = 0.314; *: holy month*

From Table 4-10, it can be observed that traffic volume in M10 was high despite the month not being of religious importance. The high traffic in M10 may be because it lies between M9 Ramadan and M11 (Dhu al-Qadah), which are months of religious importance in Saudi Arabia. This means the first week of M10 will still experience high traffic volume, as those who celebrated

Ramadan in Madinah will be travelling home during the first week of M10. The latter is also attributed to high traffic volume since people will start travelling to various religious locations to celebrate the importance of Dhu al-Qadah, a religious month in the Muslim community.

4.2.3. The Result of Ambient CO Concentrations

Carbon monoxide is considered to be a good indicator of traffic pollution. In this regard, the concentrations of CO were obtained during the study year from the only fixed-site monitoring station in the Awali location (the other four locations lacked CO data because there were no monitoring stations). The total data recorded for CO at Awali location was 354 records during the study year (24 hours/ 354 days), with no missing data. From Table 4-11, it can be noted that the median concentrations of CO during the months of M9, M11 and M12 were 2.06, 2.15 and 2.24 ppm, respectively, and were thus about 36–38 times higher CO concentrations in holy months (M9, M11, M12 and M1) than normal months. However, the intermediate CO median concentration (1.89 ppm) recorded in holy months compared to normal months confirms the previous conclusion that M1 was a transition month from holy to normal. The relatively lower values of CO concentration in M1 compared to other holy months and the wide variation exhibited might be explained by the heavy traffic in the first half of the month, and the regaining of normal traffic in the second half after the departure of pilgrims.

Furthermore, the skewness test showed that the data distribution of CO concentrations during holy months (M9, M11 and M12) varied between approximately symmetric and moderately positive skewness compared to the other months (M4–M8, M10, M1–M3), which varied between approximately symmetrical and moderate/high skewness, leading to right-tailed distribution and confirming the non-normality of data. The right-tailed skewness in the data demonstrates that more than 50 % of the data were below the mean and that the mean was greater than the median because holy months, which comprised about three and a half months (M9, M11, M12 and half of M1) of the 12 months of the study year, are associated with extremely high CO concentrations when compared to other months.

By applying the Kruskal-Wallis with H-value = 207.9 ($p<0.001$) to the CO data collected from the Awali location, as represented in Table 4-11, it was concluded that there was a noticeable difference in the CO data distribution. The Mann Whitney Test was carried out 66 times (12 months with two combinations), with a corrected Bonferroni p-value <0.004, contributing to a marked difference in the CO data distribution at the Awali location between holy, transition and normal months of the study year as denoted by different letters (a, b, c). The results of the Kruskal-Wallis and Mann Whitney Tests showed that there was a difference in the distribution of CO concentration during the Islamic year in Awali, so that the distribution of CO concentrations during the holy months (denoted by a) were statistically different from the distribution of CO concentration during the normal months (denoted by c). This was a result of the fact that, during the holy months, the Awali location experiences high traffic volume compared to normal months. The results also showed that the distribution of ambient CO concentration in M1 (denoted by b) was statistically different between holy and normal months. This is because, as mentioned earlier, the first half of Muḥarram (M1) is considered holy and the second half normal.

Table 4-11: Ambient CO concentration (ppm) at the Awali location during the study year

Month Number	Month Name	Median	Minimum	Maximum	IQR	Skewness	Distribution symmetry
M4	Rabi ath-thani[c]	1.31	1.21	1.59	0.16	0.81	+
M5	Jumada al-ula[c]	1.31	1.21	1.53	0.11	1.11	++
M6	Jumada al-akhirah[c]	1.32	1.16	1.66	0.29	0.65	+
M7	Rajab[c]	1.38	1.23	1.57	0.25	0.175	≈0
M8	Shaban[c]	1.52	1.23	1.64	0.21	0.368	≈0
M9*	Ramaḍan[a]	2.06	1.24	2.90	0.77	0.12	≈0
M10	Shawwal[c]	1.52	1.31	2.80	0.29	2.60	++
M11*	Dhu al-Qadah[a]	2.15	1.57	2.83	0.53	0.01	≈0
M12*	Dhu al-Ḥijjah[a]	2.24	1.82	2.53	0.19	0.54	+
M1*	Muḥarram[b]	1.89	1.18	2.30	0.83	-0.14	≈0
M2	Ṣafar[c]	1.31	1.21	1.57	0.13	1.01	++
M3	Rabi al-awwal[c]	1.31	1.20	1.97	0.09	3.24	++

*Months with different letters (a, b, c) were markedly different in their data distribution, IQR: Inter Quartile Range), *: holy month, ≈0: approximately symmetrical (≤0.5); +: moderate positive skewness (>0.5 and ≤1.0); ++: high positive skewness (>1.0), SES: Standard Error of Skewness = 0.427*

To further clarify, Figure 4-16 showed the box plot of CO concentrations (ppm) at the Awali location during the study year. The box plots of M9, M11 and M1 showed a wide variation of ambient CO concentrations (minimum, Q1, median, Q3 and maximum) compared to that recorded in M12 and normal months. It was obvious that the median concentrations in holy months (>2 ppm) were higher than in normal months (≤1.5 ppm). On the other hand, relatively lower values of CO concentrations were recorded during M1 compared to other holy months. This wide variation in M1 might be attributed to the resumption of normal traffic in the second half of the month after the departure of the remaining pilgrims.

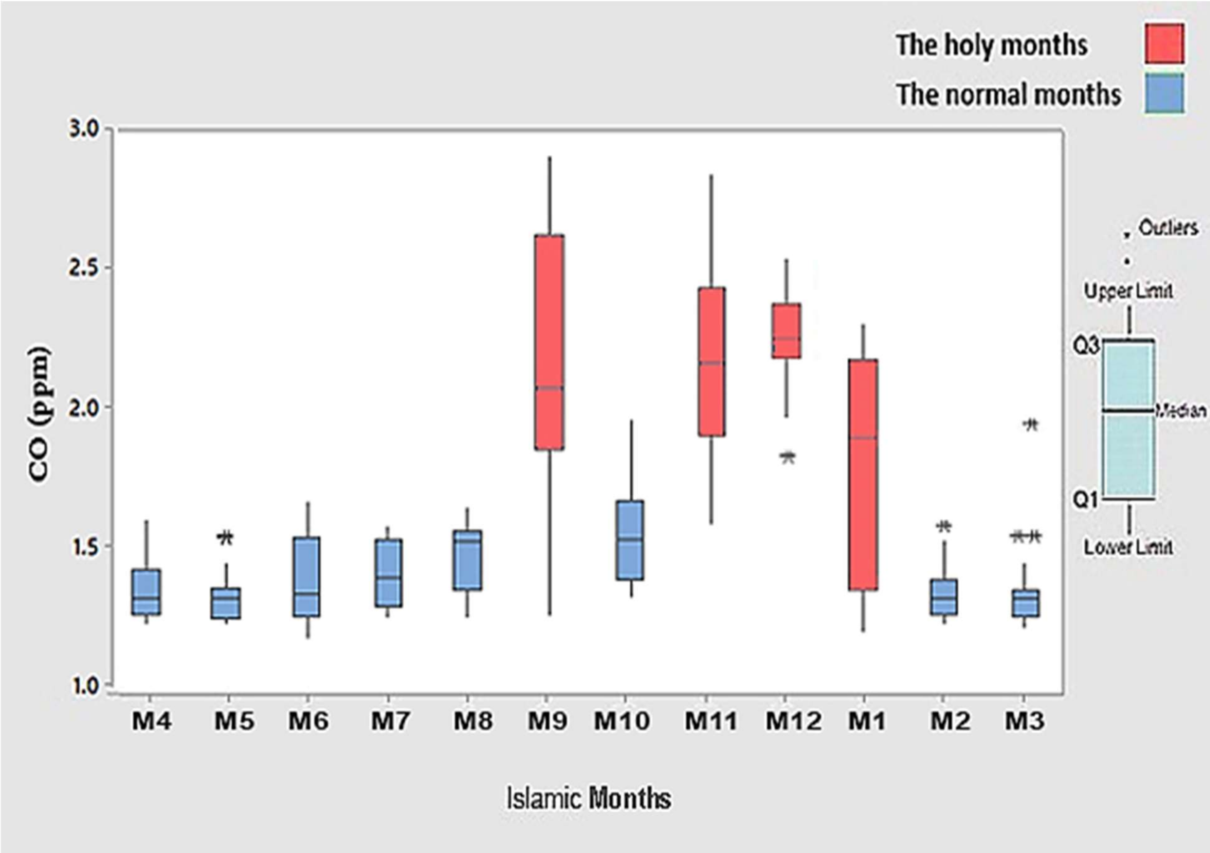


Figure 4-16: Box plot of CO concentrations (ppm) at the Awali location during the study year

The calendar plot in Figure 4-17 illustrates the CO levels during the months of the study year. The daily ambient CO concentrations are demonstrated by the red colour gradient, with the darker grade pointing to a higher concentration or hotspots corresponding to the holy months. The higher CO concentrations (>2.8 ppm) were detected in Awali during the M9 Ramadan event, the first days of M10–M11 when there was Hajj, and to slightly lower concentrations ranging between (2.4–2.6 ppm) in the following month (M12), as well as to the first half of M1 (2.0–2.4 ppm), until the departure of all pilgrims. It can be concluded that the darkest colour gradient was concentrated in the holy months with the higher traffic, while the lighter gradients were observed in the normal months with the lower traffic volume. This can be attributed to the higher transportation rates during the religious months.

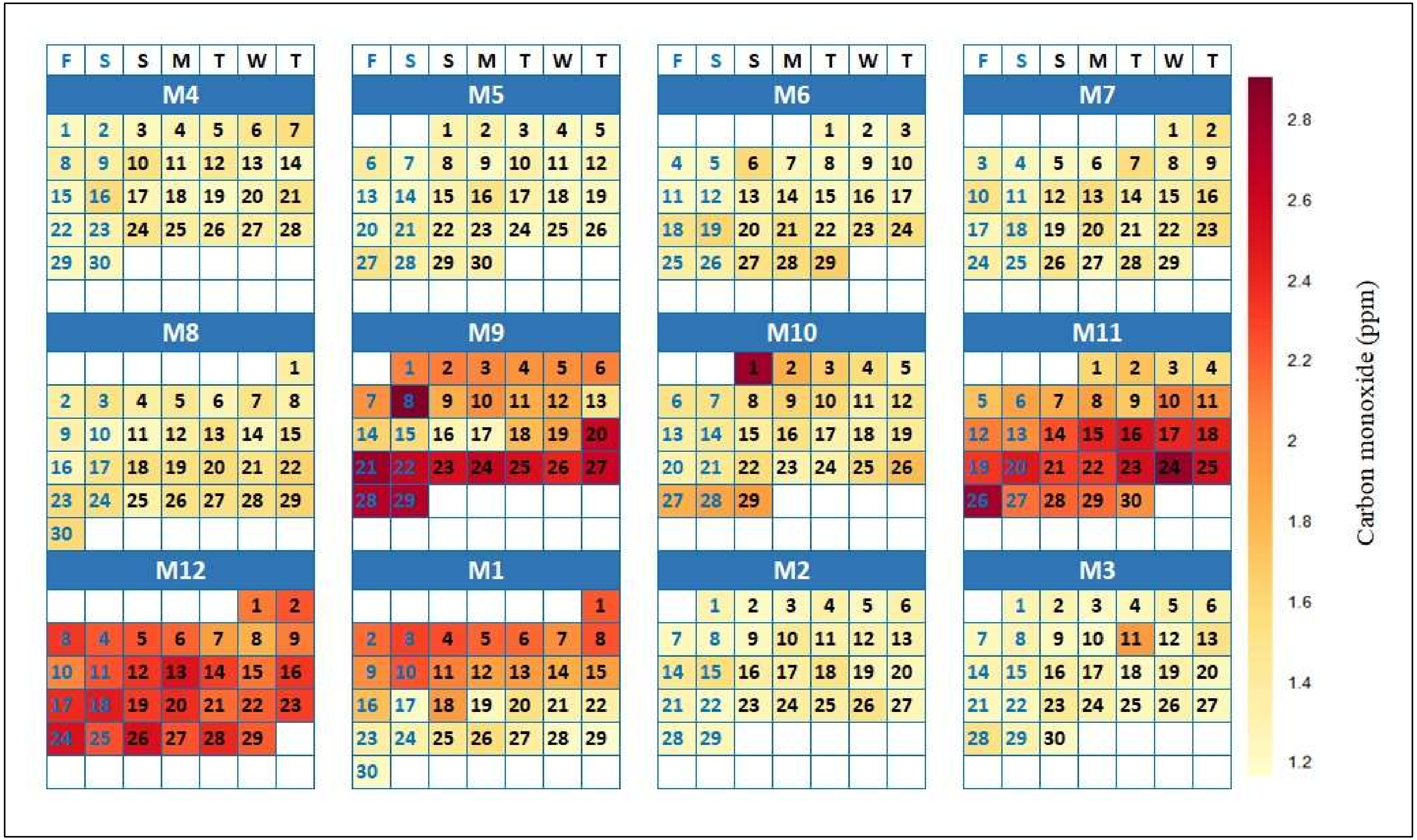


Figure 4-17: Calendar plot of daily CO concentration (ppm) in Awali during the study year

4.2.4. Comparison of CO Concentrations between Normal Months, Ramadan and Hajj Months in Awali

Median CO concentrations over 24 h for the three time periods (normal months, Ramadan and Hajj months) in Awali were demonstrated in Figure 4-18. The study hours were 16.00–23.00, to coincide with the janitors' work shift. The three time periods had almost the same median CO concentration (~1.5 ppm) between 12.00–15.00. In the normal months, the median CO concentration did not show any elevation and ranged from 1–1.5 ppm over the 24 h. A marked decrease to 0.5 ppm appeared between 02.00–04.00 (not included in the study hours). In Ramadan, median CO concentration in the Awali traffic area was found to increase above 2 ppm between 19.00–03.00, i.e. between sunset and dawn. The shift to the right in the Ramadan curve can be attributed to the different sleep pattern during Ramadan and the religious activities in these hours, leading to outdoor activities with subsequent heavy traffic in the streets. As shown in Figure 4-18, the maximum CO concentration (> 3.5 ppm) was recorded at 23.00. During Hajj, the median CO concentration in Awali followed the same pattern as in normal months, but with noticeably elevated levels above 2 ppm from 16.00–01.00, and reached ~ 3.5 ppm between 18.00–23.00.

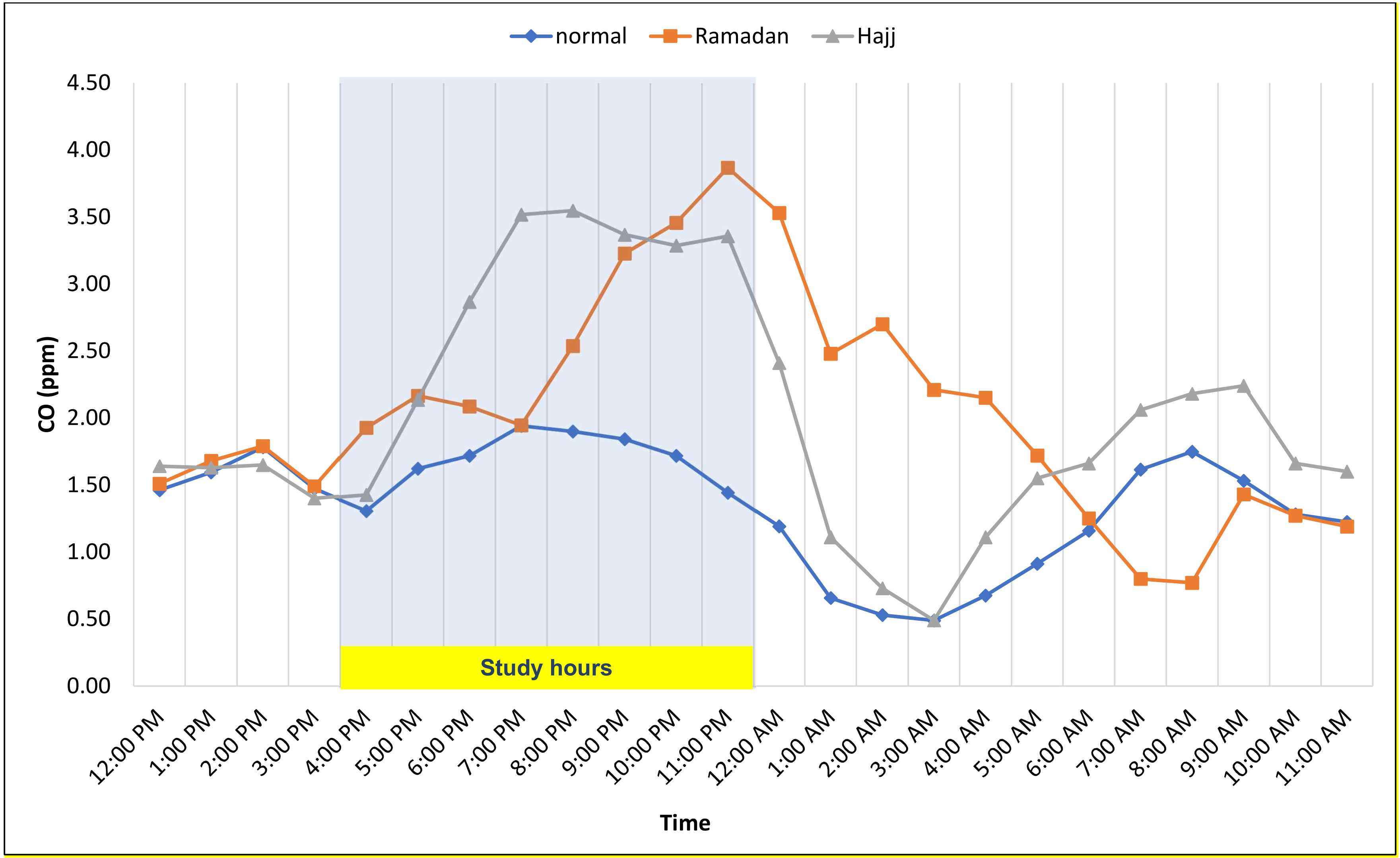


Figure 4-18: Comparison of median CO concentration (ppm) over 24h in normal, Ramadan, and Hajj months in Awali during the study year

4.2.5. Time Series of Ambient CO Concentration during the Week

In Figure 4-19, the CO concentration curves were similar for weekdays and weekends from 00.00–12.00, and then the weekend curve continued to decrease; the weekday curve flipped due to the decrease in the CO concentrations to <0.5 ppm between 01.00–03.00, and started to increase >0.5 ppm from 04.00, reaching its peak value of < 2.0 ppm at 08.00. It finally decreased again to <1.5 ppm between 09.00–11.00.

The highest levels of median ambient CO concentration (>1.5 ppm) in Awali were seen as three peaks during weekdays (07.00–09.00 and 12.00–15.00, both not included in the study) and 17.00–23.00 (included in the study). On the other hand, at weekends, median ambient CO concentrations (>1.5 ppm) were seen as two peaks at 13.00–14.00 (outside the study hours) and 16.00–23.00 (within the study hours). The lowest levels of median ambient CO concentrations (<1ppm) were between 01.00–05.00 during weekdays and 03.00–08.00 at weekends. However, similar median concentrations were recorded in the study time between 15.00–23.00. The low median concentrations are associated with the sleep hours and hours with no religious activities, while high median concentrations are associated with rush hours and religious activities. During the rush hour, traffic volume is high, resulting in high CO concentration. From Figure 4-19, it can be concluded that there was no statistically significant difference between weekdays and weekends in CO concentrations, shown by the Binary Logistic Regression Test (Appendix 7-10).

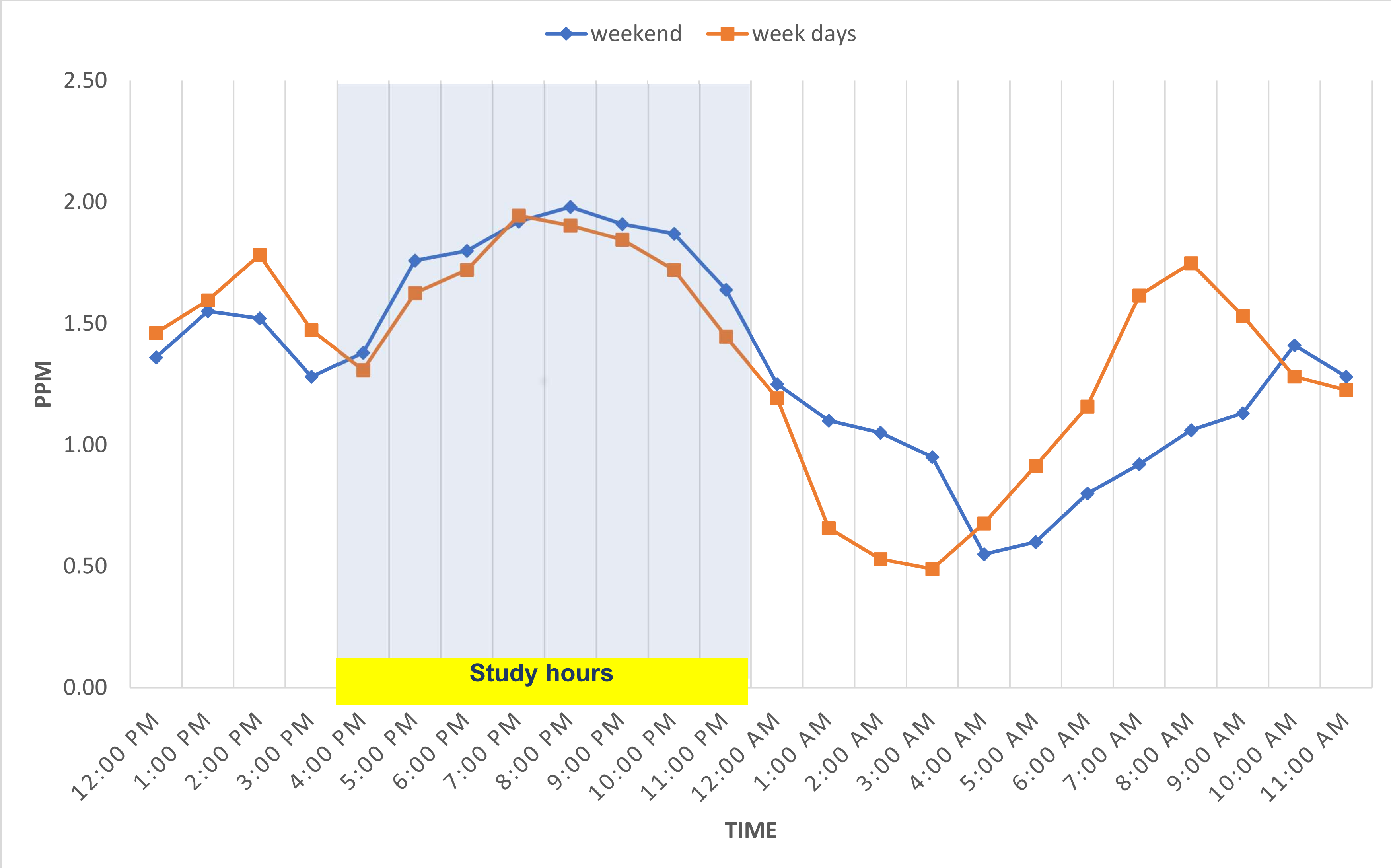


Figure 4-19: Median ambient CO concentration (ppm) of each hour of workdays and weekends in Awali, during the study year

4.3.Result of Meteorological Measurements

To understand and explain air pollution better, it was necessary to study the effect of different meteorological parameters and determine the extent to which they may affect the levels of air pollution. From this point of view, the meteorological records of Madinah were obtained from 20 stations for the whole study year (354 records/year), including temperature, relative humidity, wind speed and precipitation. The role of meteorological data was also studied in terms of the exposure to air contaminants from traffic congestion in the vicinity of the main holy place in Madinah (the Prophet's Masjid) throughout the study year. It is important to note that the meteorological data used in this study were obtained from General Authority of Meteorology and Environmental Protection, and that these data are not specific to the various sites where they were obtained, but are for Madinah in general.

Table 4-12 shows that throughout the study year the temperature in Madinah ranged between 5–49°C (median 34.4 °C), relative humidity ranged between 3–45% (median 16%), wind speed was 1–8 km/h (median 3%), and precipitation was low at 0.01–0.20 mm (median 0.06 mm). To examine the data distribution of each parameter, skewness was calculated using the median coefficient, where relative humidity was expressed as a symmetrical distribution, and wind speed and precipitation was expressed as an asymmetric right-tailed distribution with moderately and highly positive skewness, respectively. Also, the temperature expressed a left-tailed distribution with a moderately negative skewness.

Table 4-12: Meteorological data in Madinah during the study year

Variable parameters	Median	Minimum	Maximum	IQR	Skewness	Distribution symmetry
Temperature (°C)	34.40	5.00	49.05	13.8	-0.64	-
Relative Humidity (%)	16.00	3.00	45.00	12.01	0.48	≈0
Wind Speed (km/h)	3	1	8	2	0.85	+
Precipitation (mm)	0.06	0.01	0.20	0.10	6.30	++

IQR: Inter Quartile Range, ≈0: approximately symmetric (≤0.5); +: moderate positive skewness (>0.5 and ≤1.0); ++: high positive skewness (>1.0), moderate negative skewness (<-0.5 and > -1.0); SES: Standard Error of Skewness = 0.058

Repeated temperature measurements were taken daily through the study year (354 days) to plot a time series graph, as shown in Figure 4-10. Astronomical calculations showed that the Islamic calendar is shorter than the Gregorian calendar by 11 days, and so the climate shifts 11 days every new Islamic year and the cycle is repeated every 33 year; therefore, the temperature chart is specific to this Islamic calendar year. A gradual increase in temperature occurred from M4 to M8 and reached a maximum from M9 to M12, where it remained nearly constant. A downward decrease in temperature occurred between M12 and M3, without reaching the starting temperature in M4 due to the 11-day difference between Islamic and Gregorian calendar.

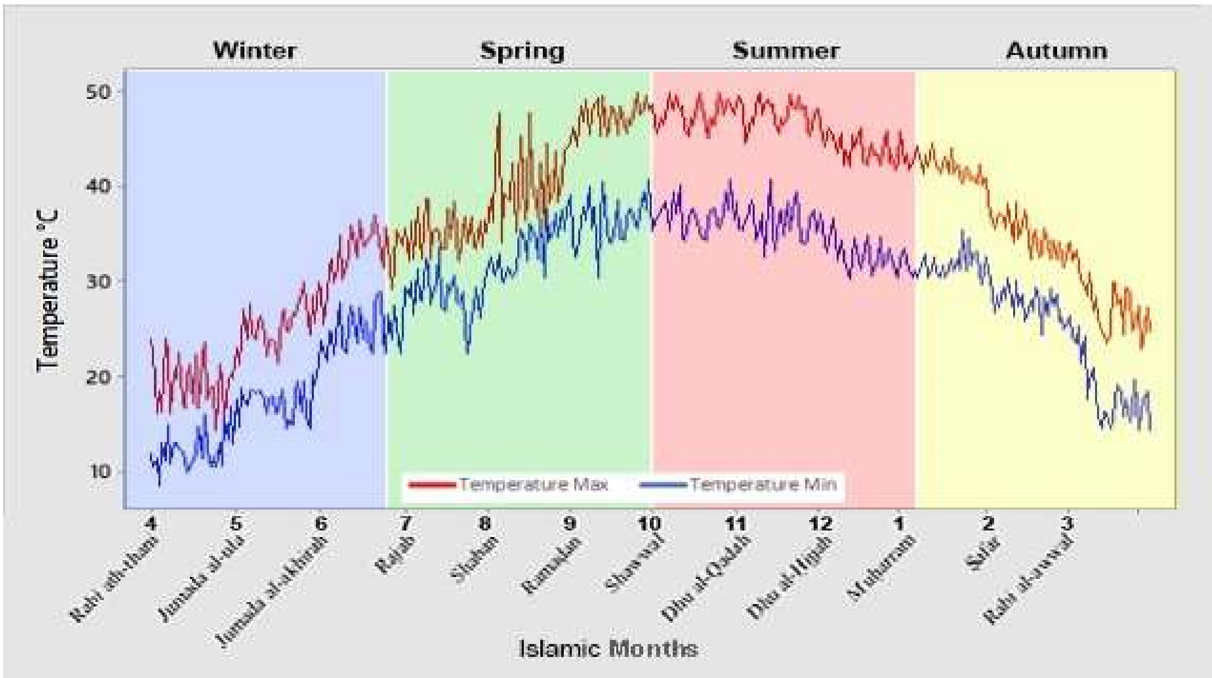


Figure 4-10 : Time series plot of daily maximum and minimum temperature recordings in Madinah during the study year

On the other hand, a windrose plot is shown in Figure 4-11 to demonstrate the wind direction and speed frequencies in Madinah throughout the study year. The data were summarized by direction, different wind speed ranges, and the percentage of time that the wind blows from a certain angle. Wind speeds split the intervals shown by the scale in each panel. The grey circles showed the percentage frequencies, and the length of each 'spoke' around the circle is related to the frequency of time that the wind blows from a particular direction. The four categories for speed are: blue for 0–2 ms-1/very low speed); green for 2– ms-1/low speed; yellow for 4–6 ms-1/medium speed; and, red for 6–8 ms-1/high speed. The direction towards the centre represents the wind direction. From the windrose plot in Figure 4-11, it was clear that the winds in Madinah were mostly coming from the northwest and the north. Wind speeds were between low and medium speed throughout the year, ranging from 2 to 6 ms^{-1}; however, in rare cases (about 5%), the wind speed was higher than 7 ms^{-1}, which is considered high speed.

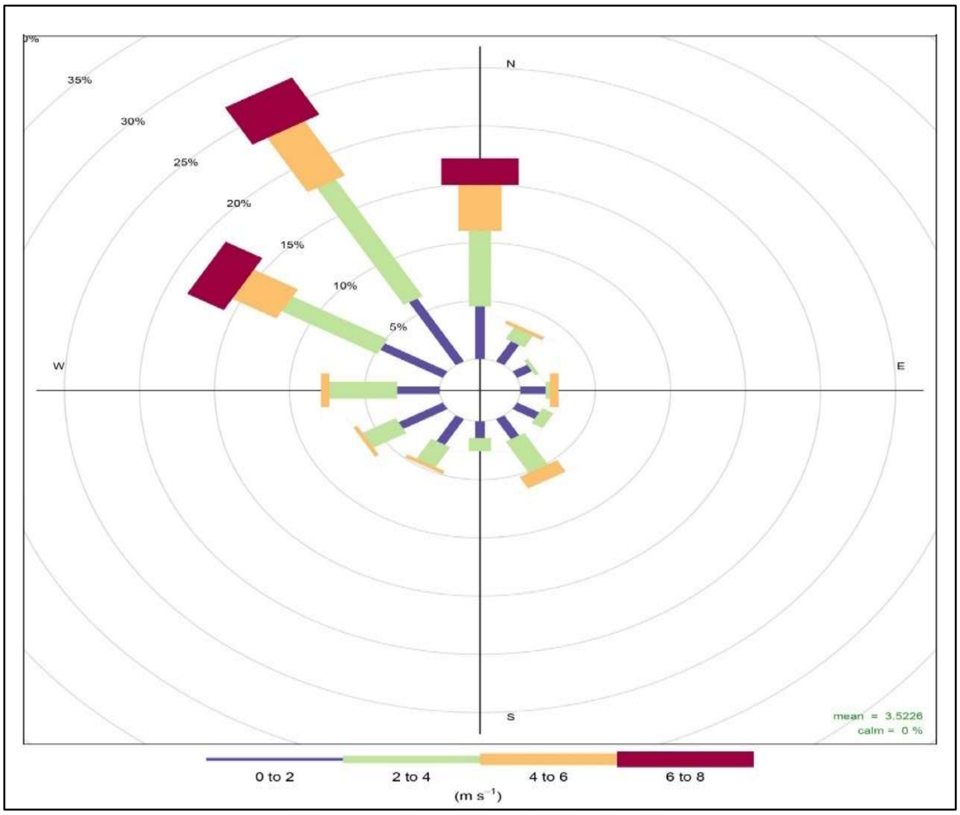


Figure 4-11: Windrose plot for wind direction/speed frequencies in Madinah during the study year

4.4.The Results of BaP Monitoring

This section will demonstrate the measurement methods used in monitoring BaP contamination in the five locations in Madinah during one Islamic calendar year excluding weekends (Friday and Saturday). In this regard, two measurement methods were used. The first determined the ambient BaP concentrations through a high-volume air sampler installed at each location and operated for 24 h, and the second aimed to determine the BaP concentrations in the breathing-zone air samples collected by the personal air samplers carried by street janitors; these were operated for eight hours according to their working hours.

4.4.1. Ambient BaP

The results of monitoring ambient BaP in samples collected from high volume air samplers installed at the five locations (Downtown, Quba, Awali, Sultana and Al-Azizyah) during one Islamic calendar year are explained in this section. Measurements were taken for each of the five locations on a daily basis excluding weekends (one sample for each location 5 days/week, as mentioned in Chapter 3). Therefore, the number of air samples collected from each location was 240 samples in the study year (one sample x 20 working days/ month x 12 months) contributing to a total number of 1,200 of samples from the five locations.

Descriptive data gave an overview of ambient BaP results represented in Table 4-13, showing minimum, maximum and mean ambient BaP concentrations as well as IQR and skewness. The median concentrations in the three traffic locations of Downtown, Quba and Awali were 0.45, 0.43 and 0.37 ng/m^3, respectively, while in the control locations of Sultana and Al-Aziziyah this was 0.02 ng/m^3. It is clear from Figure 4-12 that medians in the traffic locations were about 18–22 times higher compared to control locations, while the maximum concentrations were four times higher than the minimum in all locations except in Al-Aziziyah, where the maximum was ten times higher than the minimum.

To assess the statistical significance between the ambient BaP data distributions in the five locations, the Kruskal-Wallis Test with H-value = 882.8 ($p<0.001$) was performed, followed by ten Mann Whitney Tests (five locations and two combinations) with a Bonferroni adjusted p-value of <0.01. As represented in Table 4-13, the locations with different letters (a, b, c) (traffic locations and control locations) were distinctly different from each other in the ambient BaP data distribution. The two tests show that the distributions of ambient BaP concentrations in traffic locations are statistically different from those in control locations. The difference was brought out by the fact that traffic locations were always associated with high traffic volume and a great number of vehicles. This resulted in high BaP concentration compared to low traffic volume and a low number of vehicles in the control locations, resulting in relatively low BaP concentrations. The traffic locations in this study were: Downtown, a religious area with a high population density and narrow streets; Quba, a commercial overcrowded area; and, Awali, a commercial and residential area with narrow streets linking the south of Madinah to the city centre. These suffered the same heavy traffic and hence high BaP concentration equivalent to the religious, commercial and residential activities. On the other hand, in the control locations, Sultana, the highly-urbanized area and Al-Aziziyah, the model residential neighbourhood with low population density and no crowded streets, had very low BaP concentrations.

Furthermore, the right-skewed distribution in the data of the three traffic locations and one control location (Sultana), as well as the non-skewness of data in Al-Aziziyah, confirmed the non-normality of data resulting from the concentration of BaP in the ambient air due to the heavy activities (religious, commercial, residential) in the traffic locations and the relatively nearer distance of Sultana to traffic hot spots compared to Al-Aziziyah.

Table 4-13: Ambient BaP concentration (ng/m^3) at five locations in Madinah during the study year

Location	Median	Minimum	Maximum	IQR	Skewness	Distribution symmetry
Downtown[a]	0.45	0.21	0.84	0.13	0.760	+
Quba[a]	0.43	0.21	0.82	0.21	0.548	+
Awali[a]	0.37	0.21	0.83	0.20	0.925	+
Sultana[b]	0.02	0.01	0.10	0.02	0.502	+
Al Aziziyah[c]	0.02	0.01	0.04	0.01	0.055	≈0

Locations with different letters (a, b, c) were markedly different from each other in their data distribution, IQR: Inter Quartile Range, SES: Standard Error of Skewness= 0.309, ≈0: approximately symmetric (≤0.5); +: moderate positive skewness (>0.5 and ≤1.0).

From Table 4-13, it can be concluded that the median BaP concentrations in traffic locations were generally higher than those in control locations. On this basis, locations associated with heavy traffic can be considered hot spots for BaP exposure.

Moreover, to achieve a good indication of how the values in the data were spread out, and using minimum, first quartile (Q1), median, third quartile (Q3) and maximum values, the box plot in Figure 4-12 demonstrates the distribution of ambient BaP concentrations in the five locations. The difference in the length of plotted boxes and whiskers expresses the variation of ambient BaP concentrations. The values of BaP concentration in the traffic locations (Downtown, Awali and Quba) ranged between 0.21–0.84 ng/m^3, compared to much lower concentrations (not more than 0.1 ng/m^3) in the control locations of Sultana and Al-Aziziyah. In Awali, there was a wider variation in the upper 50% of values (upper box (Q3) and whisker) than the lower 50% of values (lower box (Q1) and whisker). This indicates the presence of relatively higher positive skewness in traffic locations compared to the control locations, where there were low and stable concentrations of ambient BaP. This was especially true of Sultana compared to Al-Aziziyah, confirming the fact that Sultana is a highly-urbanized area with many markets and café shops, while

Al-Aziziyah is a model residential area with a low population density far from pollution sources. The outliers were extremely clear and confirmed the variability of ambient BaP concentration, especially in the Downtown and Awali traffic locations and the Sultana control location.

From the previously presented data, it can be concluded that the great variation in ambient BaP concentrations is attributable to the heavy traffic in the area around Prophet Mohammed's Mosque (Downtown), with the fluctuations rising and falling according to holy events and prayer times versus normal months and different hours of day and night. After performing all the religious rites, human activities were spread but were especially close to markets (Quba location), leading to variations in traffic density and variation in ambient BaP concentrations.

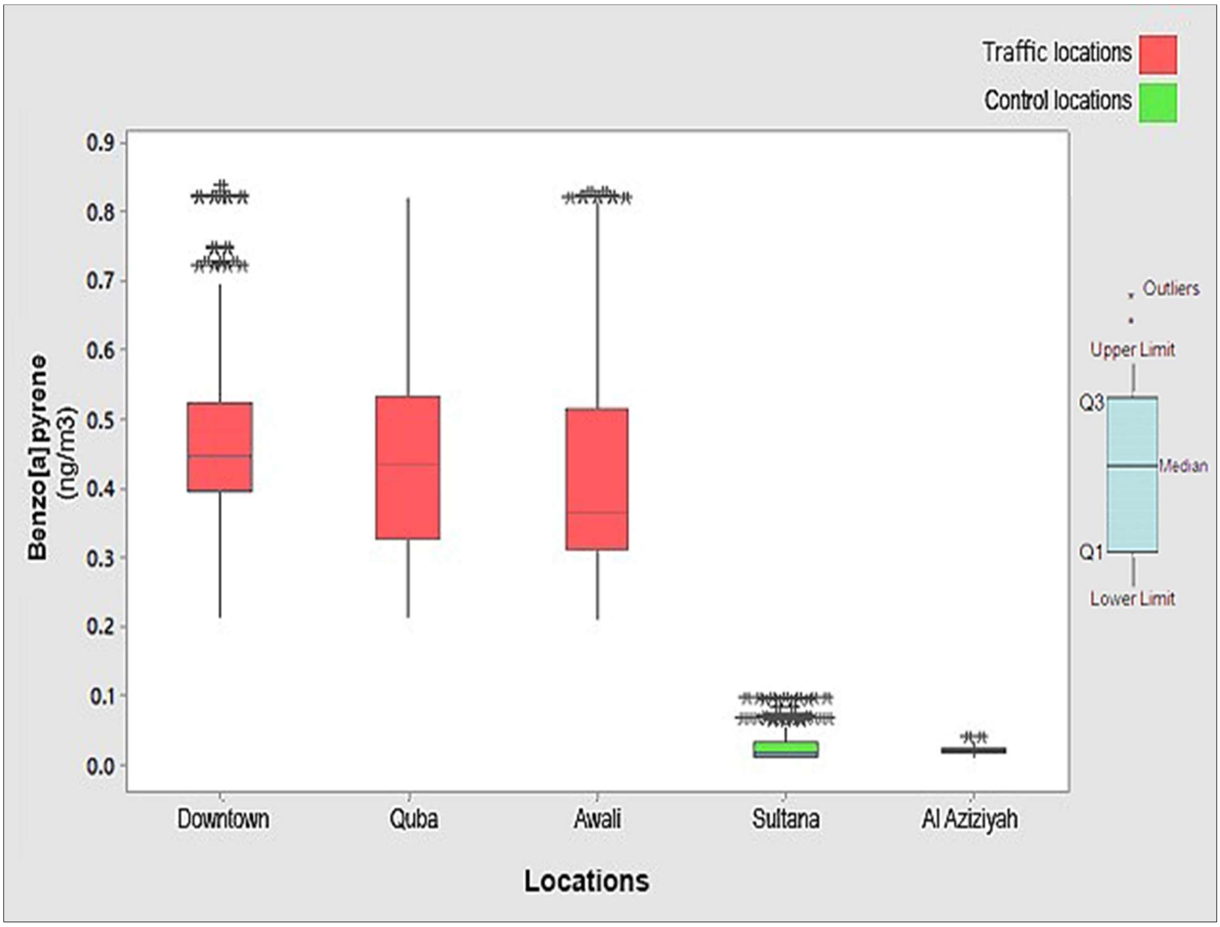


Figure 4-12: Box plot of ambient BaP concentrations (ng/m^3) at five locations in Madinah during the study year

To correlate the ambient BaP concentrations in the traffic locations with months and religious events, a calendar plot was used. This is the same as a time series chart, but the plot makes it easier to compare the approximate concentrations in different days in each month of the study to determine the hot spots having the highest BaP concentration. The calendar plot in Figure 4-13 demonstrates the daily BaP concentrations in the traffic locations on a monthly basis during the study year as a red colour gradient, of which the darkest colour represents the highest BaP concentration >0.8 ng/m^3. No data were recorded during weekends (Fridays and Saturdays), as previously mentioned. It is clear from Figure 4-13 that the hot spots with the highest BaP concentrations were distributed between M9, M11, and M12, but there were also a few hot spots in the first half of M1. From the previous observations, a strong relationship between religious events and high BaP concentrations can be concluded and vice versa in the transition month M1. In this month, the high BaP concentration was only recorded until the departure of all pilgrims and then the levels started to return back to its normal values.

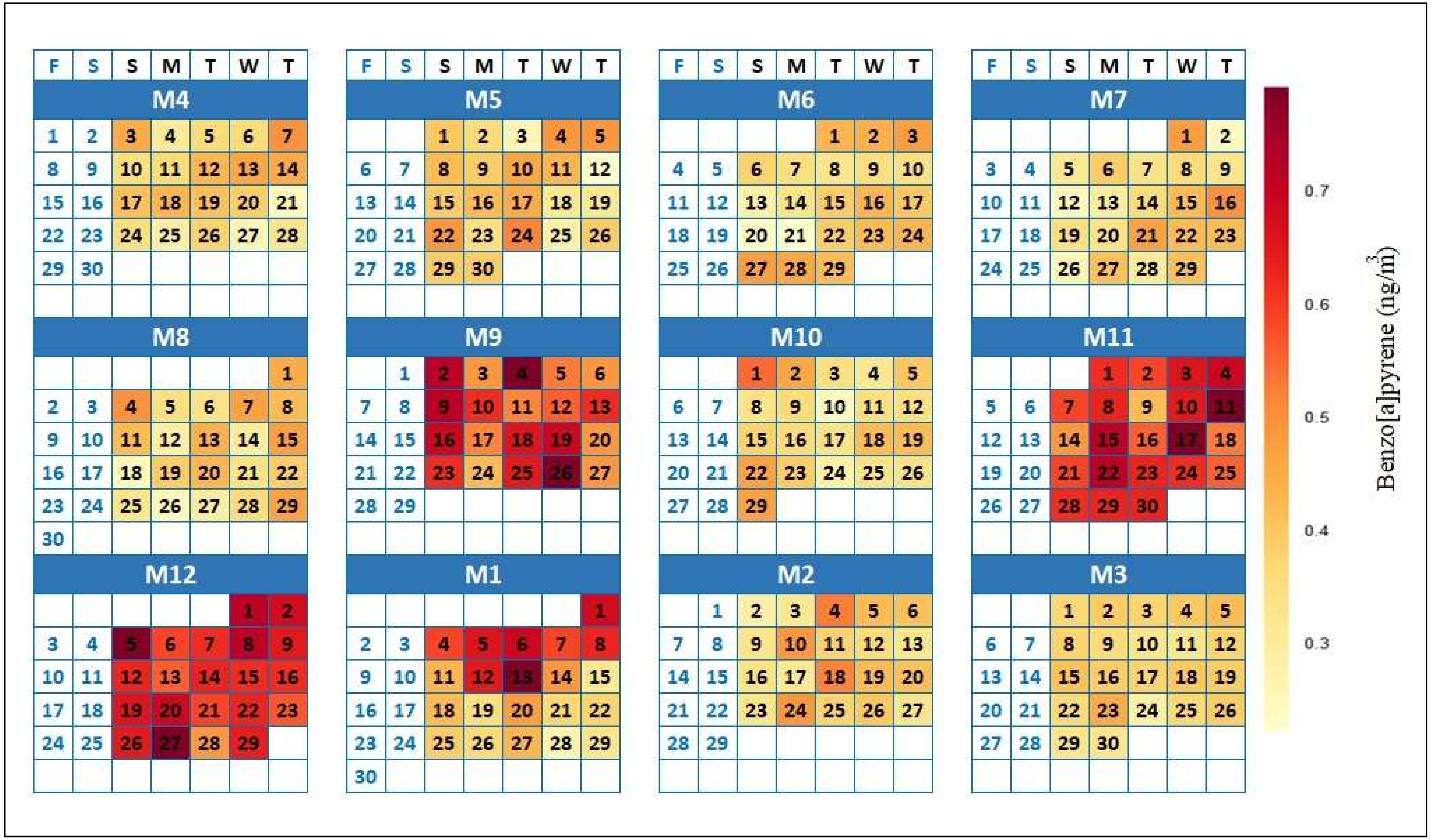


Figure 4-13: Calendar plot of daily ambient BaP concentration (ng/m^3) per month in traffic locations during the study year

Furthermore, to study in depth the levels of BaP in the traffic locations on a monthly basis, as mentioned earlier in Chapter 3, three ambient air samples were collected daily from the three traffic locations, with a total of 60 samples per month (3 samples x 20 working days/ month). This contributed 720 samples during the study year for the determination of ambient BaP contamination. Descriptive data for ambient BaP per month of the study year are summarized in Table 4-14, from which we can observe that the ambient BaP concentrations in the normal months ranged between 0.36–0.39 ng/m3 and increased to 0.59 ng/m3 in M9 due to Ramadan, and reached the maximum (0.63 ng/m^3) in M11 and M12 due to Hajj. With the departure of most of the pilgrims in the second half of M1, the median concentration started to decrease (0.43 ng/m^3) until it reached its normal level (0.39 ng/m^3) in M2 with the departure of all pilgrims.

Moreover, the data distribution was approximately symmetrical around its median during the months of the study year since the skewness ranged between $^-0.5$ and 0.5 ($^-0.5 \leq$ skewness $\leq$ 0.5). The skewness in the data confirms that the holy months (M9, M11, M12 and most of M1) were associated with extremely high BaP concentrations, ranging between 0.27–0.79 ng/m^3 compared to other normal months (M2–M8 and M10), which ranged between 0.22–0.53 ng/m^3.

From the statistical analysis of applying the Kruskal-Wallis Test with H-value = 249.7 ($p<0.001$), a marked difference can be seen between the data distributions of normal months and holy months throughout the study year, except for one month (M1) which was statistically different from both normal and holy months. After applying 66 Mann Whitney Tests (12 months with two combinations) with a Bonferroni corrected p-value <0.004, it was concluded that there was marked a difference between distributions of ambient BaP in the traffic locations during the holy months (a), transition month (b), and normal months (c), throughout the study year. This difference, according to the Kruskal-Wallis and Mann Whitney tests, is statistically significant. The medians of BaP concentrations represented in Table 4-14 showed a marked difference between holy months

(M9, M11–M12) and normal months (M4–M8, M10, M2–M3), whereas the BaP median in M1, as a transition month, differed from both holy and normal months because it was affected in its first half by holy months and in its second half by normal months. The traffic locations in the holy months marked (a) had relatively higher ambient BaP concentrations (0.59–0.63 ng/m^3) compared to the normal months marked (c), which had relatively lower ambient BaP concentrations. This can be attributed to the high population capacity and high traffic volume in holy months, and religious events such as Ramadan and Hajj. During these periods, diesel engine buses transport visitors and pilgrims, contributing to a significant rise in ambient BaP concentrations compared to normal months, when there is lower population density (no visitors or pilgrims) and hence lower traffic volume.

Table 4-14: Ambient BaP concentrations (ng/m^3) at traffic locations during months of the study year

Month no.	Month Name	Median	Minimum	Maximum	IQR	Skewness	Distribution symmetry
M4	Rabi ath-thani[c]	0.38	0.25	0.50	0.08	0.111	≈0
M5	Jumada al-ula[c]	0.39	0.22	0.52	0.09	-0.224	≈0
M6	Jumada al-akhirah[c]	0.39	0.24	0.48	0.10	0.096	≈0
M7	Rajab[c]	0.36	0.25	0.50	0.10	0.202	≈0
M8	Shaban[c]	0.39	0.25	0.50	0.10	0.117	≈0
M9*	Ramaḍan[a]	0.59	0.42	0.79	0.13	-0.052	≈0
M10	Shawwal[c]	0.36	0.25	0.56	0.08	0.229	≈0
M11*	Dhu al-Qadah[a]	0.63	0.43	0.79	0.07	-0.003	≈0
M12*	Dhu al-Ḥijjah[a]	0.63	0.43	0.79	0.08	-0.090	≈0
M1*	Muḥarram[b]	0.43	0.27	0.79	0.23	0.269	≈0
M2	Ṣafar[c]	0.39	0.29	0.53	0.08	-0.153	≈0
M3	Rabi al-awwal[c]	0.38	0.29	0.50	0.06	-0.041	≈0

*Months with different letters (a, b, c) were markedly different in their data distribution, IQR: Inter Quartile Range, *: holy month, ≈0: approximately symmetric (≤0.5); SES: Standard Error of Skewness = 0.309*

Representing the data in Table 4-14 with a box plot as in Figure 4-14, the variation in the minimum, maximum and median BaP concentrations were demonstrated by the length of boxes and whiskers. The high variation in the BaP concentration recorded during M9 was represented by the relatively long boxes and whiskers, while the very short boxes and whiskers corresponding to M3 contributed to least variation in ambient BaP concentrations.

The same conclusion was drawn from the box plot in Figure 4-14, as the medians of ambient BaP concentration (ng/m^3) in M9, M11 and M12 were similar to and higher than normal months and the medians of normal months were also similar. They were also not different to M1, which was unique as its box plot resembles the maximum value for the concentrations of holy months and its minimum value the concentrations of normal months.

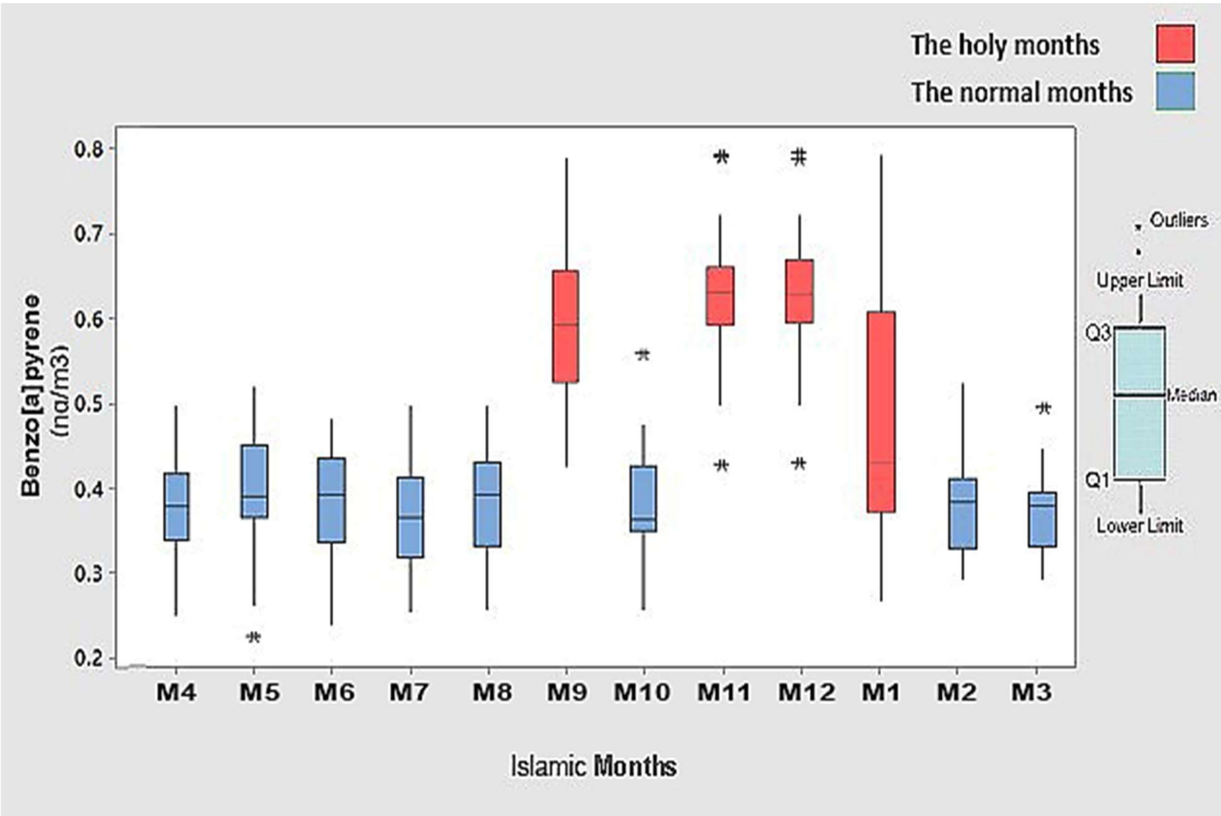


Figure 4-14: Box plots of ambient BaP concentrations (ng/m^3) at traffic locations during months of the study year

Similarly, it was necessary to assess the levels of ambient BaP in the control locations. Two ambient air samples were collected daily from the two control locations, a total of 40 samples per month (two samples x 20 working days/month), or 480 samples during the study year.

The descriptive analysis for the results is summarized in Table 4-15 to evaluate ambient BaP air contamination. Data showed high/moderate positive skewness throughout all months. Although a stringent p-value ($p<0.001$) was used in applying Kruskal-Wallis Test with H-value = 100.2, it was concluded that there was no marked difference between the distribution of BaP data in the control locations throughout the holy and normal months of the study year, and hence all months were denoted by the same letter (a). There were no marked differences in the maximum ambient BaP concentrations of M11 and M12 (0.06 ng/m^3) compared to the maximum concentration recorded in M6 (0.04 ng/m^3). Ambient BaP concentrations in the control locations were very low and ranged between 0.01 and 0.06 ng/m^3, where the median concentration was 0.02 ng/m^3, confirming that ambient BaP concentration in the two control locations throughout the months of the study year were similar.

Table 4-15: Ambient BaP concentration (ng/m^3) at control locations during the study year

Month no.	Month Name	Median	Minimum	Maximum	IQR	Skewness	Distribution symmetry
M4	Rabi ath-thani[a]	0.02	0.01	0.04	0.02	1.5	++
M5	Jumada al-ula[a]	0.02	0.01	0.05	0.01	1.7	++
M6	Jumada al-akhirah[a]	0.02	0.01	0.04	0.01	2.0	++
M7	Rajab[a]	0.02	0.01	0.05	0.02	1.3	++
M8	Shaban[a]	0.02	0.01	0.05	0.01	1.3	++
M9*	Ramaḍan[a]	0.02	0.02	0.05	0.01	1.1	++
M10	Shawwal[a]	0.02	0.01	0.06	0.02	1.4	++
M11*	Dhu al-Qadah[a]	0.02	0.01	0.06	0.01	0.9	+
M12*	Dhu al-Ḥijjah[a]	0.02	0.02	0.06	0.01	0.9	+
M1*	Muḥarram[a]	0.02	0.01	0.06	0.01	1.4	++
M2	Ṣafar[a]	0.02	0.01	0.06	0.01	1.7	++
M3	Rabi al-awwal[a]	0.02	0.01	0.05	0.01	1.3	++

*IQR: Inter Quartile Range, +: moderate positive skewness (>0.5 and ≤1.0); ++: high positive skewness (>1.0), SES: Standard Error of Skewness = 0.374, *: holy month.*

For the visual representation of BaP data for holy and normal months in both traffic and control locations, a time series was plotted (Figure 4-15). The ambient BaP concentrations at traffic locations exhibited modal distribution and the data points exhibited two clear peaks during holy months compared to normal months, reflecting the fact that the BaP levels were not affected in the control locations during the holy months.

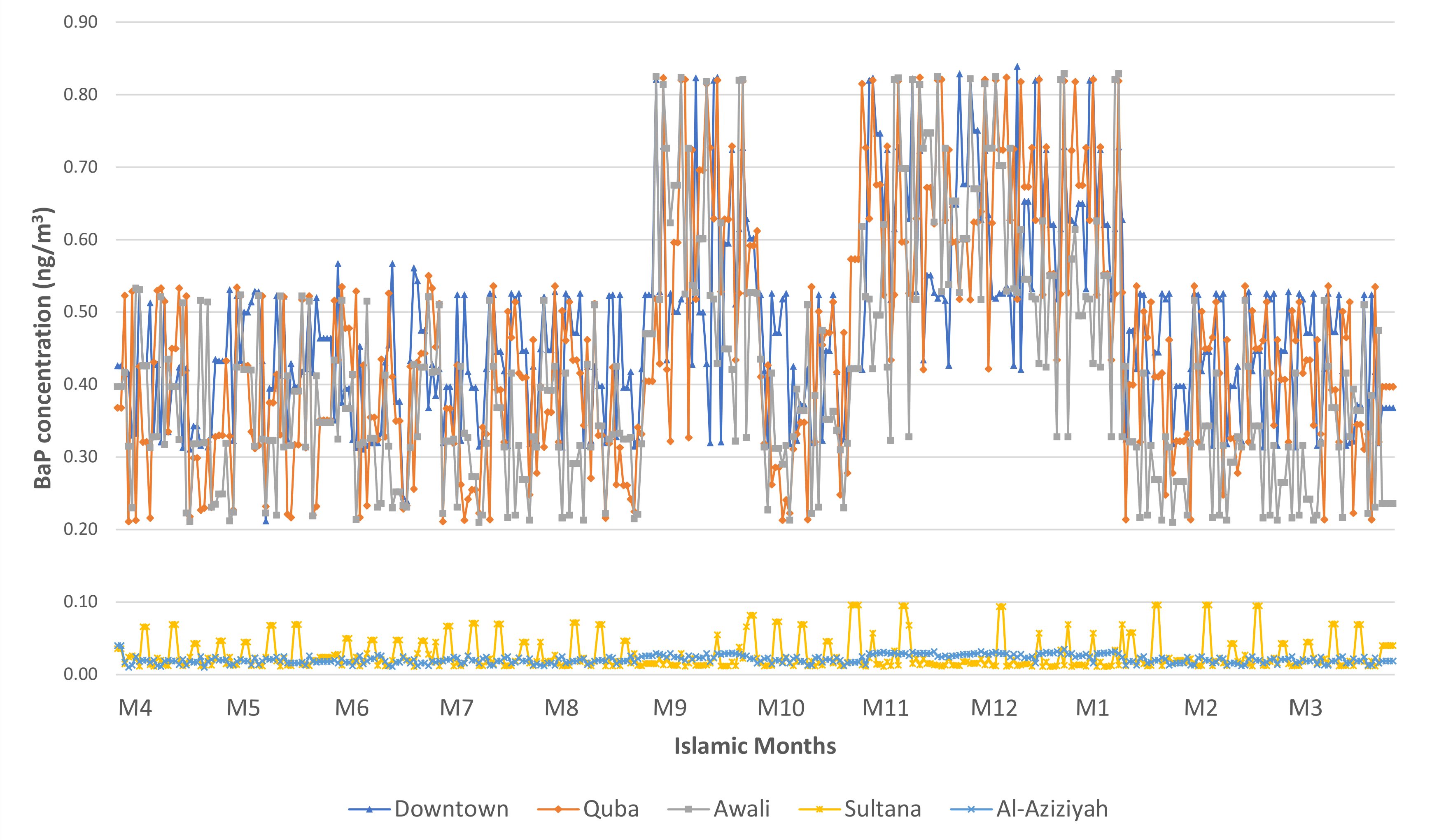


Figure 4-15: Time series plot of ambient BaP concentration (ng/m^3) at five locations in Madinah during the study year

4.4.2. Work-Related Individual Exposure to BaP

This section presents the results of measuring the work-related individual BaP in air within the breathing zone of the participants (street janitors) during an 8-hour work shift. One air sample was collected daily (except weekends) from one participant holding a personal air-sampler device at each of the five study locations in Madinah. Therefore, the number of personal air samples collected from each location was 240 samples throughout the study year (one sample x 20 working days/ month x 12 months), contributing to a total number of 1,200 of samples from the five locations.

Table 4-16: Individual BaP concentration (ng/m^3) for street janitors at five study locations during the study year

Location	Median	Minimum	Maximum	IQR	Skewness	Distribution Symmetry
Downtown[a]	0.35	0.13	0.54	0.13	0.159	≈0
Quba[a]	0.37	0.22	0.56	0.11	0.016	≈0
Awali[a]	0.35	0.12	0.57	0.13	13.7	++
Sultana[b]	0.02	0.01	0.05	0.01	0.731	+
Al Aziziyah[b]	0.02	0.01	0.03	0.01	-0.217	≈0

Locations with different letters (a, b) were markedly different from each other in their data distribution, IQR: Inter Quartile Range, ≈0: approximately symmetric (≤0.5); +: moderate positive skewness (>0.5 and ≤1.0); ++: high positive skewness (>1.0), SES: Standard Error of Skewness = 0.157

Table 4-16 shows that the median individual BaP concentration in traffic locations was about 16 times as high as in the control locations, where it exhibited low variability (0.35–0.37 ng/m^3), with a minimum 0.12 ng/m^3 and maximum 0.57 ng/m^3. This corresponds to a median of 0.02 ng/m^3 in the control locations with a minimum of 0.01 ng/m^3 and maximum of 0.05 ng/m^3. Both Awali and Sultana exhibited high and moderate positive skewness, respectively, while the individual BaP concentrations were approximately symmetrical in Downtown, Quba and Al-Aziziyah locations. The existence of the skewness showed that data were not normally distributed and the median values were markedly different. This skewness might be due to the fact that holy months are associated with extremely high BaP concentrations compared to other months.

Applying the Kruskal-Wallis Test with H-value = 865.9 ($p<0.001$) to the data of the individual BaP concentration for the street janitors at the five locations during the study year, it was concluded that the data distributions of the three traffic locations were distinctly different from that of the two control locations. Comparing the median individual BaP concentrations in control areas with those of the traffic areas, it was found that the distributions of data in both locations were statistically different and contributed to more BaP pollution for the janitors working in traffic areas than for those working in the control areas. Hence, individual BaP concentrations in traffic areas were different than in control areas.

Ten Mann Whitney Tests were carried out (five locations with two combinations) and p-value was adjusted with a Bonferroni correction < 0.01. Locations denoted by different letters (a, b) were noticeably different from each other. Both the Mann Witney and Kruskal-Wallis tests confirmed each other, as they showed that the distribution of individual BaP concentrations in traffic locations were statistically different from their distribution in the control locations. The per capita for janitors working in traffic areas from BaP pollution was also greater than for janitors working in control locations, and this may require more intensive health care for these persons or a frequent turn-around cycle, so that janitors in control locations switch to traffic locations and vice versa, giving a better chance for their bodies to remove contaminants.

The box plot in Figure 4-16 demonstrates that the concentrations of individual BaP in the five locations confirmed that the median concentrations in the three traffic locations were markedly higher than in control locations, having concentration values of 0.12–0.57 ng/m^3. Also, there was great variation in the individual BaP concentrations in traffic locations in the four quartiles. This variation in the traffic locations, as represented by the long whiskers, indicates that there were time periods where BaP concentrations were either higher or lower than the average daily normal BaP concentration. In contrast, the concentration values in control locations were very low and close, and almost invariably ranged from 0.01–0.05 ng/m^3. This showed that the concentration of BaP in control locations varied little with time, due to the distance of these locations from the city and traffic volume where the maximum air pollution exists.

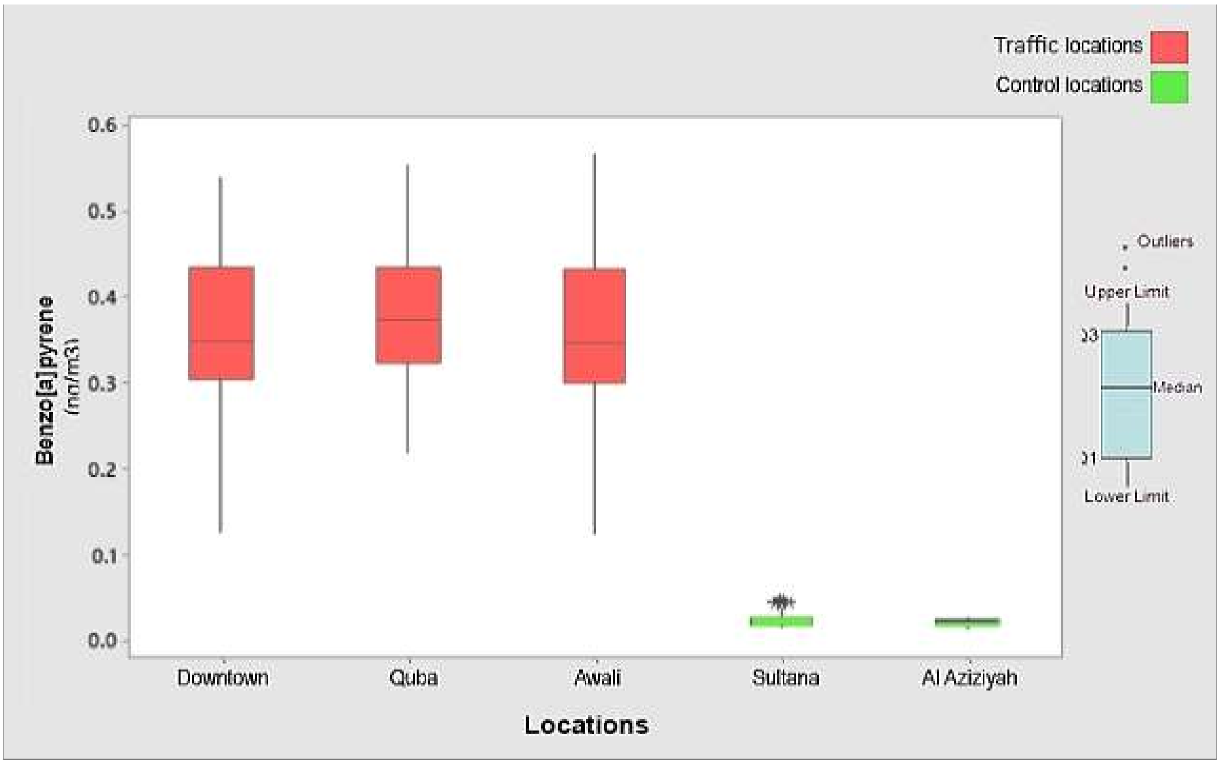


Figure 4-16: Box plot of individual BaP concentration (ng/m^3) at five locations during the study year

By demonstrating the previous individual BaP results on a calendar plot, as in Figure 4-17, the daily individual BaP concentrations appear as a gradient in a darker red colour, pointing to high concentrations (> 0.5 ng/m3) on this day. In traffic locations, high BaP concentrations (> 0.5 ng/m^3) had accumulated in M9, M11 and M12, as well as the first half of M1, corresponding to the holy months with religious events such as Ramadan and Hajj. Most, if not all, the days of M9 (Ramadan), M11 and M12 (Hajj) are deep red, indicating the highest individual BaP concentrations of 0.45–0.5 ng/m^3 for janitors working in traffic locations.

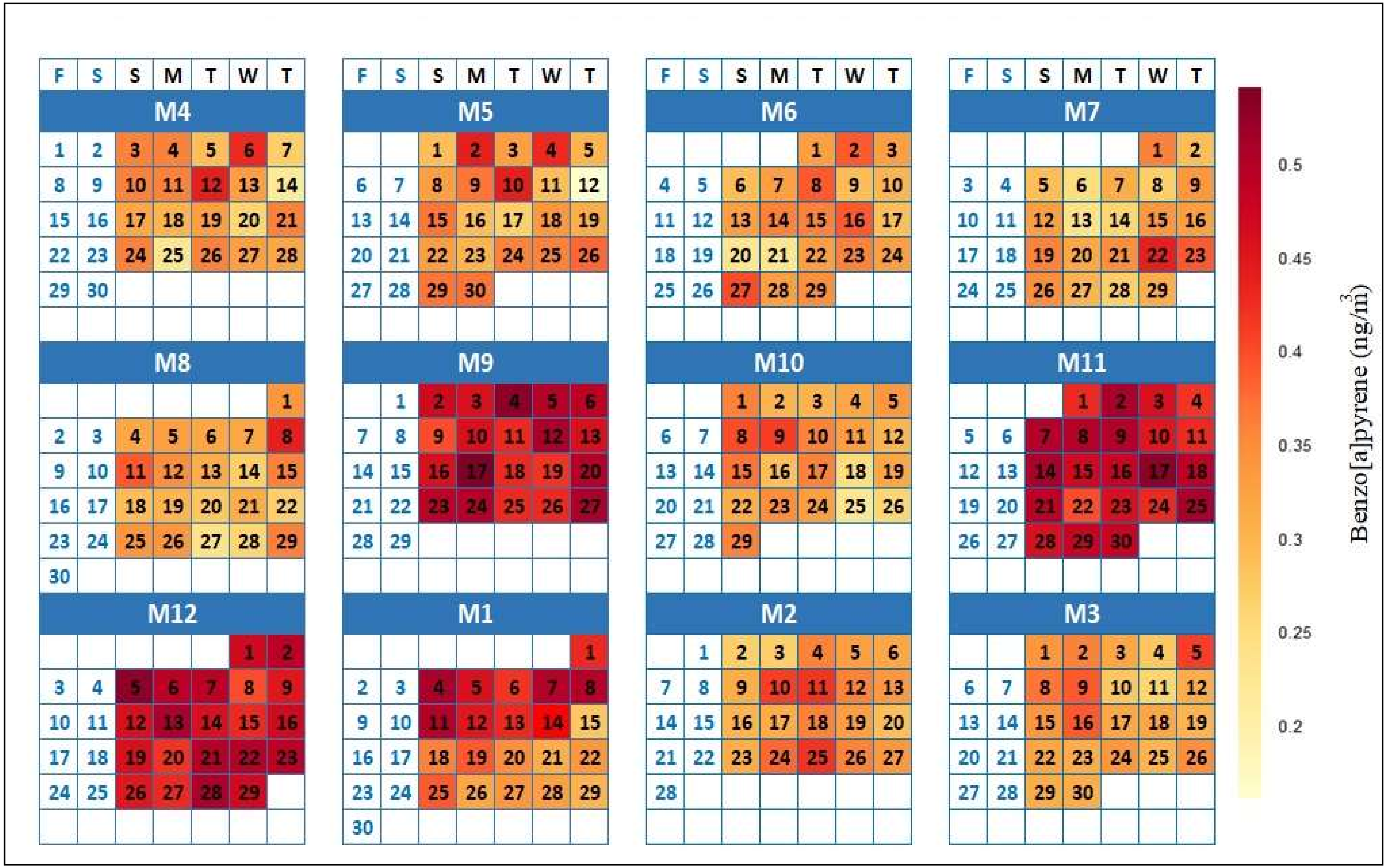


Figure 4-17: Calendar plot of daily individual BaP concentrations (ng/m^3) in traffic locations during the study year

The descriptive data of the individual BaP concentrations throughout the months of the study year are summarized in

Table 4-17. Sixty samples were collected monthly from the three traffic locations, contributing to a total number of 720 samples during the study year. The data confirmed that the traffic locations exhibited high levels, reaching maximum concentrations in M9, M11 and M12 (0.54, 0.53, 0.53 ng/m3, respectively), and a minimum concentration in M5 (0.16 ng/m^3). By using the Kruskal-Wallis Test with H-value = 258.6 ($p<0.001$), it was concluded that the months differed markedly in their data distribution, and so 66 Mann Whitney tests (12 months with two combinations) were carried out with a Bonferroni p-value correction (< 0.004).

Table 4-17: Individual BaP concentration (ng/m^3) at traffic locations during the study year

Month no.	Month Name	Median	Minimum	Maximum	IQR	Skewness	Distribution symmetry
M4	Rabi ath-thani[c]	0.35	0.22	0.44	0.06	-0.13	≈0
M5	Jumada al-ula[c]	0.33	0.16	0.44	0.07	-0.28	≈0
M6	Jumada al-akhirah[c]	0.33	0.23	0.40	0.06	1.10	++
M7	Rajab[c]	0.32	0.24	0.44	0.06	0.20	≈0
M8	Shaban[c]	0.32	0.24	0.44	0.05	0.21	≈0
M9*	Ramaḍan[a]	0.46	0.36	0.54	0.06	-0.68	-
M10	Shawwal[c]	0.33	0.24	0.41	0.05	0.16	≈0
M11*	Dhu al-Qadah[a]	0.47	0.40	0.53	0.03	-0.71	-
M12*	Dhu al-Ḥijjah[a]	0.47	0.40	0.53	0.04	-0.71	-
M1*	Muḥarram[b]	0.39	0.28	0.51	0.14	7.60	++
M2	Ṣafar[c]	0.33	0.27	0.42	0.04	-0.10	≈0
M3	Rabi al-awwal[c]	0.32	0.26	0.41	0.05	0.04	≈0

*Months with different letters (a, b, c) were markedly different in their data distribution, IQR: Inter Quartile Range, *: holy month, ≈0: approximately symmetric (≤0.5); ++: high positive skewness (>1.0); moderate negative skewness (<-0.5 and > -1.0), SES: Standard Error of Skewness = 0.309*

The statistical analysis using the Kruskal-Wallis and Mann Whitney tests for the individual BaP data in the traffic locations, such as Awali, indicated that the distribution of individual BaP concentration in the holy months (Ramadan, Dhu al-Qadah, Dhu al-Ḥijjah), denoted by (a), were statistically different from the distribution of individual BaP concentrations in the normal months (c). This difference was a result of high traffic volume in the holy months compared to normal months since the concentration of BaP is positively related to traffic volume.

On the other hand, the data distribution of individual BaP in Muḥarram (b) was also statistically different from its distribution in holy and normal months. The reason, as explained earlier, was that M1 was a transition month, with its first half resembling holy months while its second half resembled normal months. As a result, the median individual BaP concentration in the first half of M1 was high, resembling holy months, and its second half relatively low, as recorded in normal months. M1 thus showed an intermediate median of individual BaP (0.39 ng/m^3) which fell between concentrations recorded in holy months (0.47 ng/m^3) and those recorded in normal months (0.33 ng/m^3).

As can been seen in the box plot illustrated in Figure 4-18, M9, M11 and M12 were markedly higher than normal months. M1 is a mixed or transition month, showing a wide variation of concentrations in its first half, with minimum concentrations resembling values in normal months and maximum concentrations approaching that of holy months. The first half of M1 had similar characteristics to holy months and the second was similar to normal months, as represented by the wide IQR (the long distance between Q2 and Q3). The variations in individual BaP concentrations were similar to normal months. The existence of a wider variation in the median individual BaP concentration in M1 was because the first half of the month is associated with religious activities, resulting in high traffic and high individual BaP concentration. The second half of the month is associated with normal activities, resulting in low individual BaP concentration.

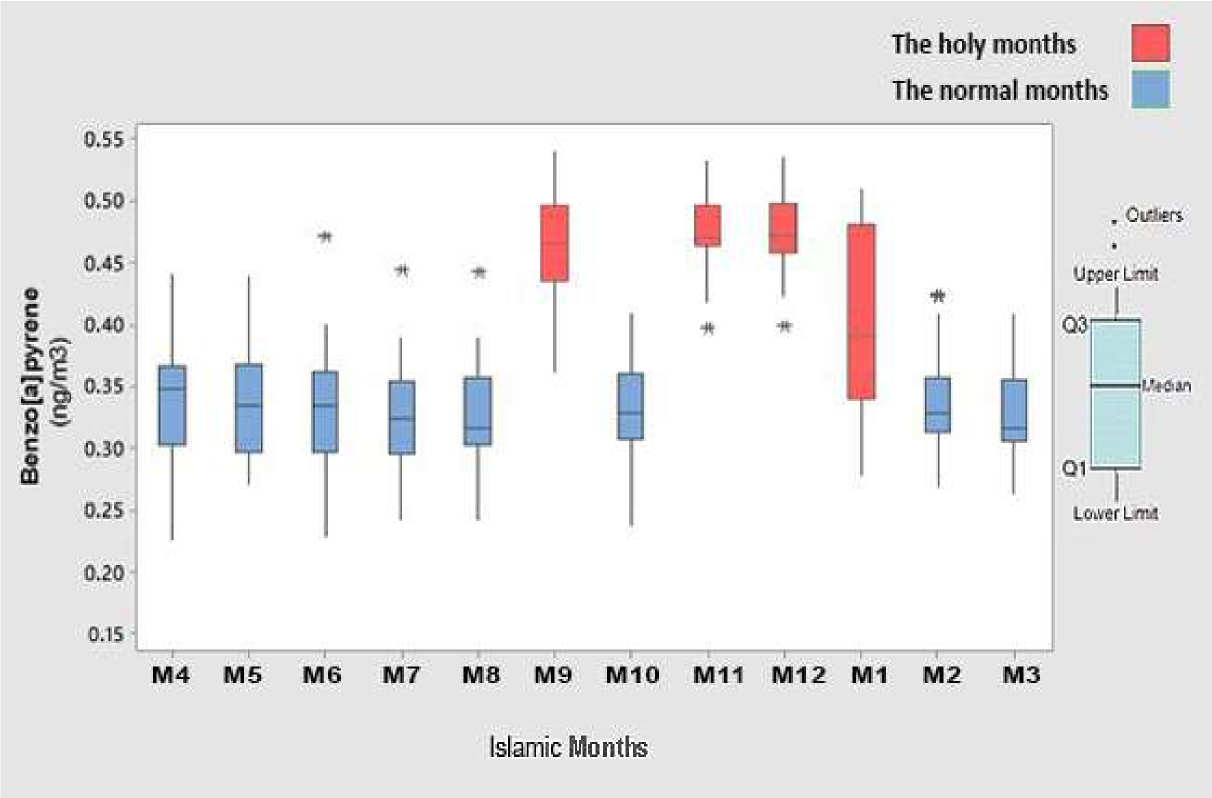


Figure 4-18: Box plot of individual BaP concentration (ng/m^3) in traffic locations during the study year

Furthermore, the descriptive data (median, minimum and maximum concentrations) of individual BaP at the control locations are summarized in Table 4-18. Forty samples were collected monthly from the two control locations, contributing to a total number of 480 samples during the study year.

Table 4-18: Individual BaP concentration (ng/m^3) at control locations during the study year

Month no.	Month Name	Median	Minimum	Maximum	IQR	Skewness	Distribution symmetry
M4	Rabi ath-thani[a]	0.02	0.01	0.03	0.00	0.03	≈0
M5	Jumada al-ula[a]	0.02	0.01	0.03	0.00	0.12	≈0
M6	Jumada al-akhirah[a]	0.02	0.02	0.04	0.00	0.33	≈0
M7	Rajab[a]	0.02	0.02	0.03	0.00	-0.18	≈0
M8	Shaban[a]	0.02	0.02	0.04	0.01	0.18	≈0
M9*	Ramaḍan[a]	0.03	0.02	0.03	0.01	0.64	+
M10	Shawwal[a]	0.02	0.02	0.04	0.01	-0.18	≈0
M11*	Dhu al-Qadah[a]	0.03	0.02	0.03	0.00	0.22	≈0
M12*	Dhu al-Ḥijjah[a]	0.03	0.02	0.03	0.01	0.24	≈0
M1*	Muḥarram[a]	0.03	0.02	0.03	0.01	0.69	+
M2	Ṣafar[a]	0.02	0.02	0.03	0.00	-0.41	≈0
M3	Rabi al-awwal[a]	0.02	0.02	0.03	0.00	-0.39	≈0

*IQR: Inter Quartile Range, *: holy month, ≈0: approximately symmetric (≤0.5); +: moderate positive skewness (>0.5 and ≤1.0), SES: Standard Error of Skewness = 0.374*

In the Kruskal-Wallis Test with H-value =102.0 ($p<0.001$), there was no marked difference between the individual BaP data distributions at the control locations, and so the null hypothesis cannot be rejected. Individual BaP scores recorded low medians of 0.02–0.03 ng/m^3, with no marked difference among all months, even holy months.

The difference between median individual BaP concentrations observed monthly in traffic and control locations is demonstrated in the time series plot in Figure 4-19. In the control locations, the concentrations during the whole year were approximately zero, while in the traffic locations they fell around 0.2 to just above 0.5 ng/m^3. This can be attributed to the high levels of ambient BaP during the holy months due to high population capacity associated with high transportation rates.

Figure 4-19: Time series plot of individual BaP concentration (ng/m^3) at five locations in Madinah during the study year

4.5. The Relation between Bap and the Most Influential Factors

In this section, the relationship between the various data collected and the conclusions drawn were discussed. Spearman's correlation analysis, scatter plots and graphs were used to determine these relationships. The correlations between individual BaP, ambient BaP, carbon monoxide, daily number of vehicles, temperature, relative humidity, wind speed and precipitation (especially the correlation between meteorological conditions and BaP concentration) were determined to understand how these factors are related.

4.5.1. Correlations between Air Pollutants, Traffic, and Meteorological Variables

The correlation between the different variable parameters are shown in Table 4-19. A significant positive correlation was observed between the number of vehicles and the following conditions: individual BaP, ambient BaP, and carbon monoxide. A significant positive correlation was also observed between carbon monoxide, individual BaP and ambient BaP, as well as between the ambient and individual BaP. In addition to this, a significant positive correlation was noted between both wind speed and carbon monoxide concentration and humidity, and between precipitation and humidity. The results also indicated a significant negative correlation between humidity and wind speed and mean temperature. A negative relationship was also noted between carbon monoxide concentration and wind speed. No statistically significant relationship was found between wind speed and BaP, however, and indeed this was not expected. A negative correlation was expected between wind speed and BaP concentration, potentially because the wind speed in Madinah varies little, making its effect on the BaP concentration negligible.

Table 4-19: Correlations between different parameters at the five locations in Madinah during the study year

Parameters	Individual BaP	Ambient air BaP	Carbon monoxide	Daily Number of Vehicles	Mean of Temperature	Humidity	Wind Speed	Precipitation
Individual BaP Benzo[a]pyrene (ng/m^3)	1	0.79**	0.60**	0.85**	0.19 ns	-0.10 ns	-0.16 ns	-0.01ns
Ambient air BaP Benzo[a]pyrene (ng/m^3)		1	0.65**	0.74**	0.20 ns	-0.13 ns	-0.12ns	0.01ns
Carbon monoxide CO (ppm)			1	0.88**	0.18 ns	-0.12 ns	-0.73 **	0.01ns
Daily Number of Vehicles (n)				1	0.23 ns	-0.18 ns	-0.03ns	0.01ns
Mean Temperature (°C)					1	-0.66**	-0.33 ns	0.09 ns
Humidity (%)						1	-0.56**	0.65**
Wind Speed (km/h)							1	0.11 ns
Precipitation (mm)								1

***: Spearman's correlation is significant (p-value < 0.01, 2-tailed), ns: correlation is not significant (p-value >0.05, 2-tailed), N= 1770*

4.5.2. The Relation between Ambient BaP Concentration and Individual BaP Concentration

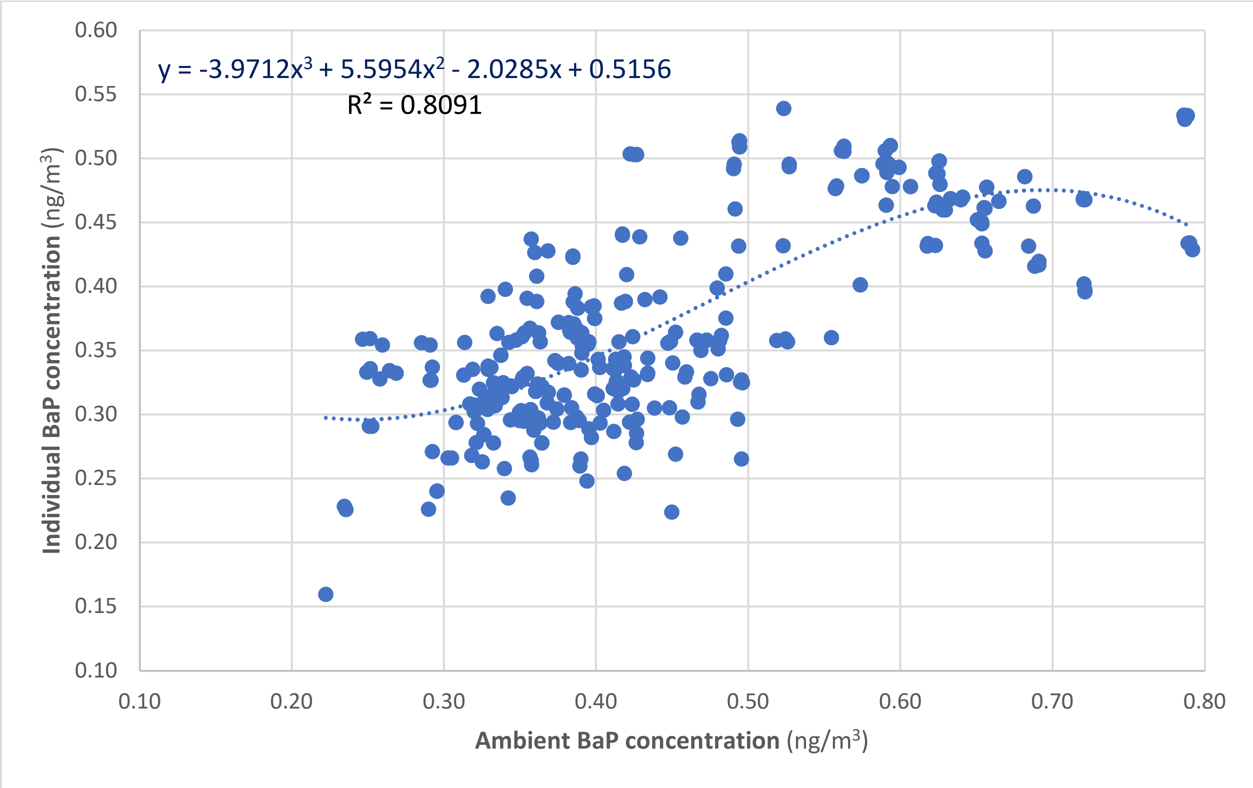


Figure 4-20: Scatter plot of the ambient BaP concentration and individual BaP concentration

As shown in

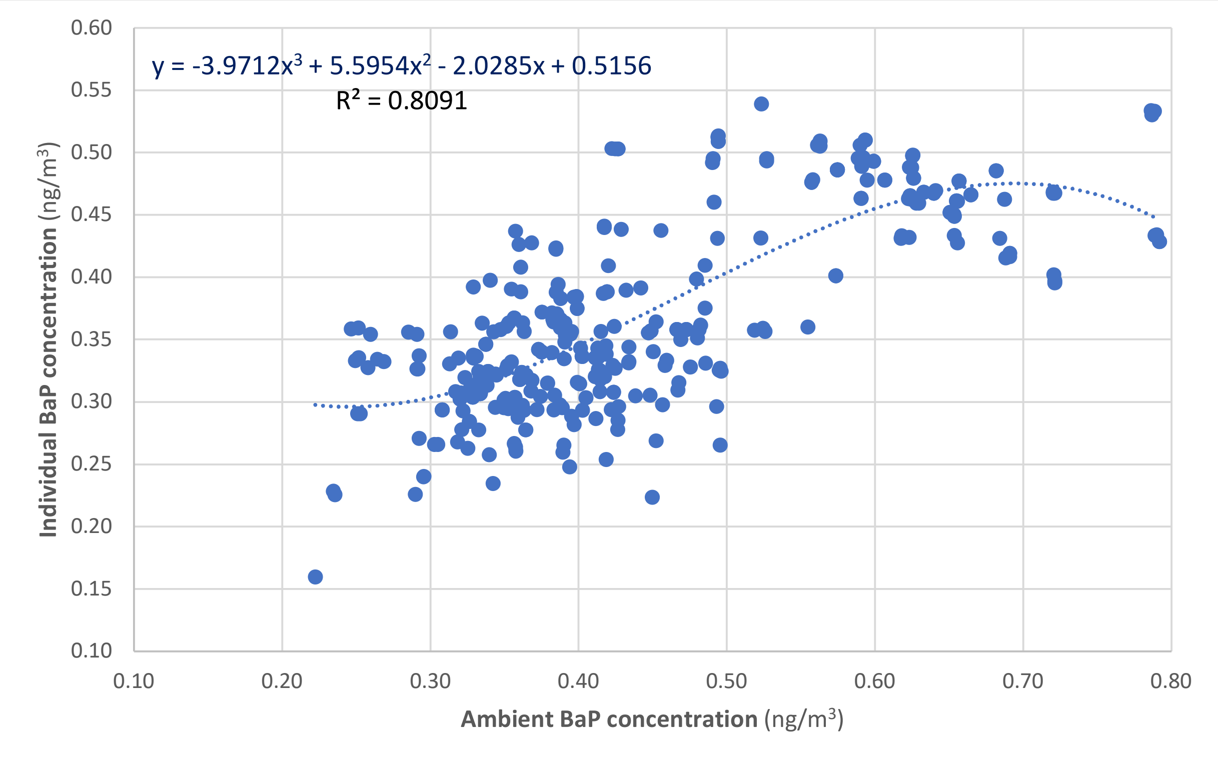
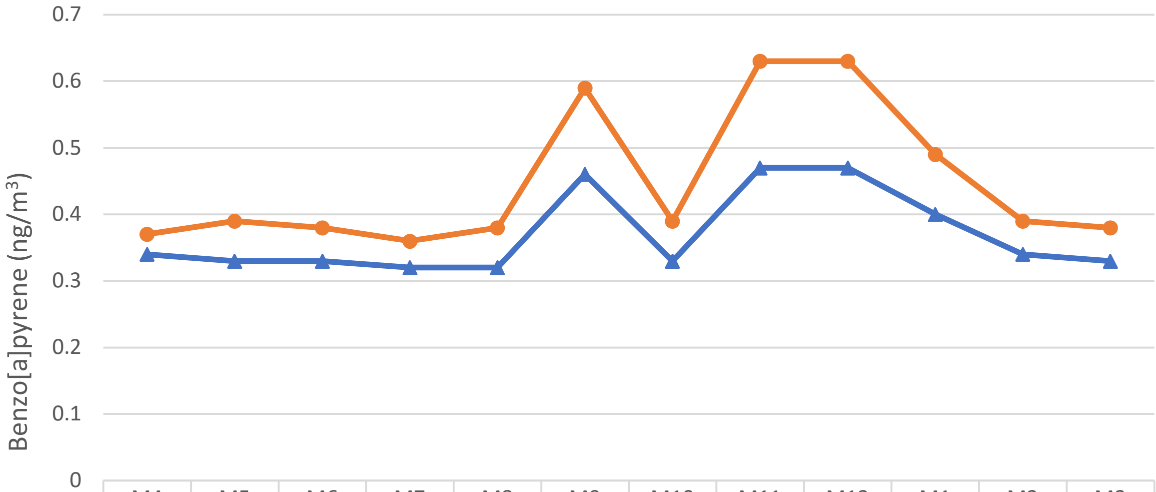


Figure **4-20**, a polynomial cubic regression model provides the best approximation of the relationship between the ambient and individual BaP, as demonstrated by the high value of R^2 = 0.8091. The Spearman's correlation test in Table 4-19 also confirms a highly significant positive correlation between the ambient BaP and individual BaP concentration (Spearman's correlation coefficient (R^2) =0.79, $p<0.001$)). The close correlation between the ambient BaP and individual BaP concentration showed that there was a positive relationship between the qualities of the ambient air actually inhaled by people in Madinah with respect to BaP concentration. The relationship between the ambient BaP (measured at a fixed station) and individual BaP (measured using a portable sampler, so measurement was taken at different locations) can help to compare the exposure of people based in a fixed location and those who are mobile with regards to BaP as an air contaminant.

4.5.3. Comparison between Median Concentrations of Individual and Ambient BaP during the Study Year

Figure 4-21 illustrates the comparison between medians of ambient and individual ambient BaP concentrations in Madinah. The figure shows there was a positive relationship between individual and ambient BaP, such that a lower concentration of ambient BaP corresponds to a lower concentration of the individual BaP, and a higher concentration of ambient BaP corresponds to a higher concentration of the individual BaP. This result is also confirmed by Table 4-19, which shows the correlation analysis. It is also observed that the line graph of the eight-hour individual BaP median fluctuates with the 24 h ambient BaP. Individual BaP had lower levels throughout the year and was almost parallel to ambient BaP, with no crossed lines, confirming the increase in air pollution levels with BaP during the holy months compared to normal months. Even though ambient BaP concentration was more than individual BaP concentration, the pattern shows a close relationship, and the results show that the quality of ambient air determines the quality of air people inhale in Madinah. Therefore, if quality of ambient air is bad in terms of high BaP concentration, the quality of individual BaP (which includes both outdoor and indoor concentrations) actually inhaled was also bad in terms of BaP concentration. Since the concentration of individual BaP (measured using a portable sampler) was lower than the ambient concentration (measured at fixed locations) throughout the year, it can be concluded that individuals located in fixed locations in high traffic areas are likely to be more exposed to BaP than mobile individuals.



	M4	M5	M6	M7	M8	M9	M10	M11	M12	M1	M2	M3
IB	0.34	0.33	0.33	0.32	0.32	0.46	0.33	0.47	0.47	0.4	0.34	0.33
AB	0.37	0.39	0.38	0.36	0.38	0.59	0.39	0.63	0.63	0.49	0.39	0.38

Islamic Months

Figure 4-21: Comparison between median individual BaP (IB) and ambient Bap (AB) concentrations (ng/m^3) during the months of the study year

4.5.4. Relation between CO and Ambient BaP in Awali

The correlation between the concentration of CO and ambient BaP was positive, as shown in Figure 4-22. The polynomial cubic regression line has an R^2 of 0.66. Therefore, it can be confirmed that CO and ambient BaP are indeed related. Spearman's correlation analysis results, in Table 4-19, also confirmed this relationship.

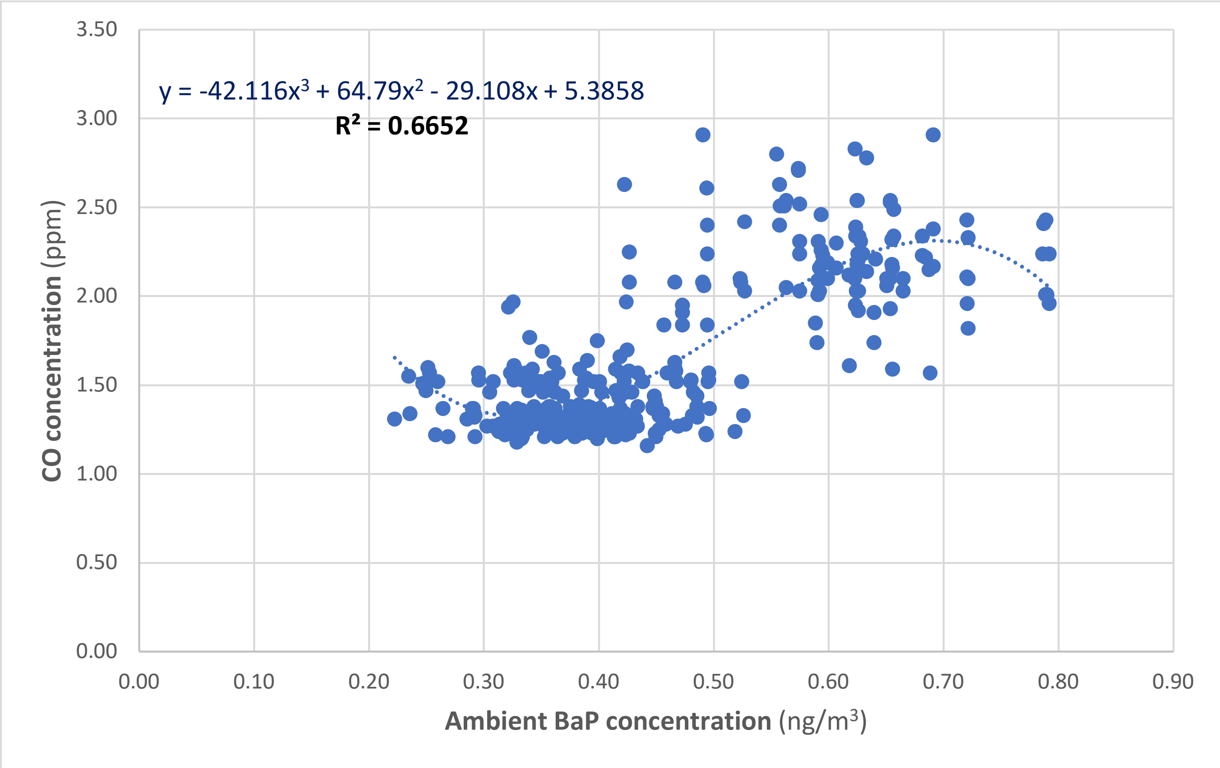


Figure 4-22: Linear regression of the relation between CO and ambient BaP concentrations in Awali

4.5.5. Relation between Ambient BaP Concentration and Traffic Volume During the Study Year

Figure 4-23 shows a time series comparison between ambient BaP concentration and traffic volume in the traffic locations during one Islamic calendar year. From the figure, it can be observed that there was a positive relationship between traffic concentration and ambient BaP concentration. Spearman's correlation analysis results in Table 4-19 also confirm this relationship. This relationship was such that a lower traffic concentration corresponds to a lower ambient BaP concentration, and a higher traffic concentration corresponds to a higher ambient BaP concentration. It was also observed that months of religious importance (holy months) (M9, M11 and M12) were associated with high traffic volume and ambient BaP concentrations (Spearman's correlation coefficient (r^2) =0.74, $p<0.001$). Both traffic volume and ambient BaP concentrations in other months remained fairly constant. The reason for the sudden increase in BaP concentration during the months of M9, M11 and M12 was that these are holy months in the Islamic calendar, and so the number of people travelling during these months was higher than those travelling in the normal months. This resulted in high traffic volumes and consequently high BaP concentrations during the holy months compared to the normal months.

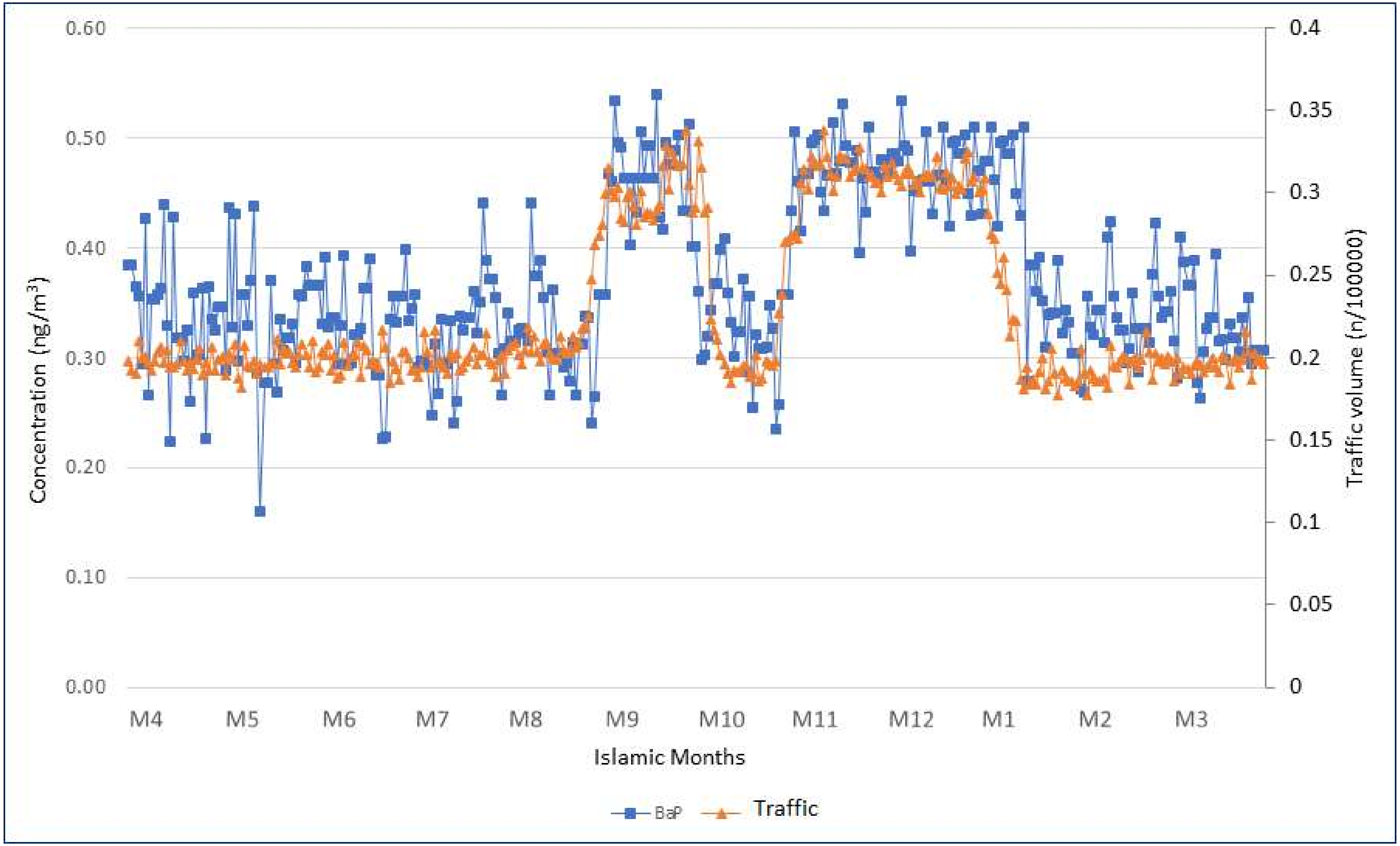


Figure 4-23: Time series plot to compare ambient BaP concentration and traffic volume in the traffic locations during one year

4.5.6. Relation Between CO Concentration and Traffic Volume During the Study Year

Figure 4-24 is a time series plot comparing ambient CO concentration and traffic volume during one Islamic calendar year. From the figure, it can be seen that there is a positive relationship between CO concentration and traffic volume, such that a higher traffic volume corresponds to a higher CO concentration, and a lower traffic volume corresponds to a lower ambient CO concentration. Also, during M9, M11 and M12, traffic volume was high and so were the CO concentrations (Spearman's correlation coefficient (r) =0.88, $p<0.001$). Traffic volume and CO concentrations in other months remained fairly constant. The relationship between traffic volume and CO is brought about by the fact vehicular emissions contain CO, and therefore an increase in traffic is accompanied by an increase in CO concentration. The reason for the sudden increase in traffic volume and CO during M9, M11 and M12 is that these Muslim holy months have high numbers of people travelling compared to normal months.

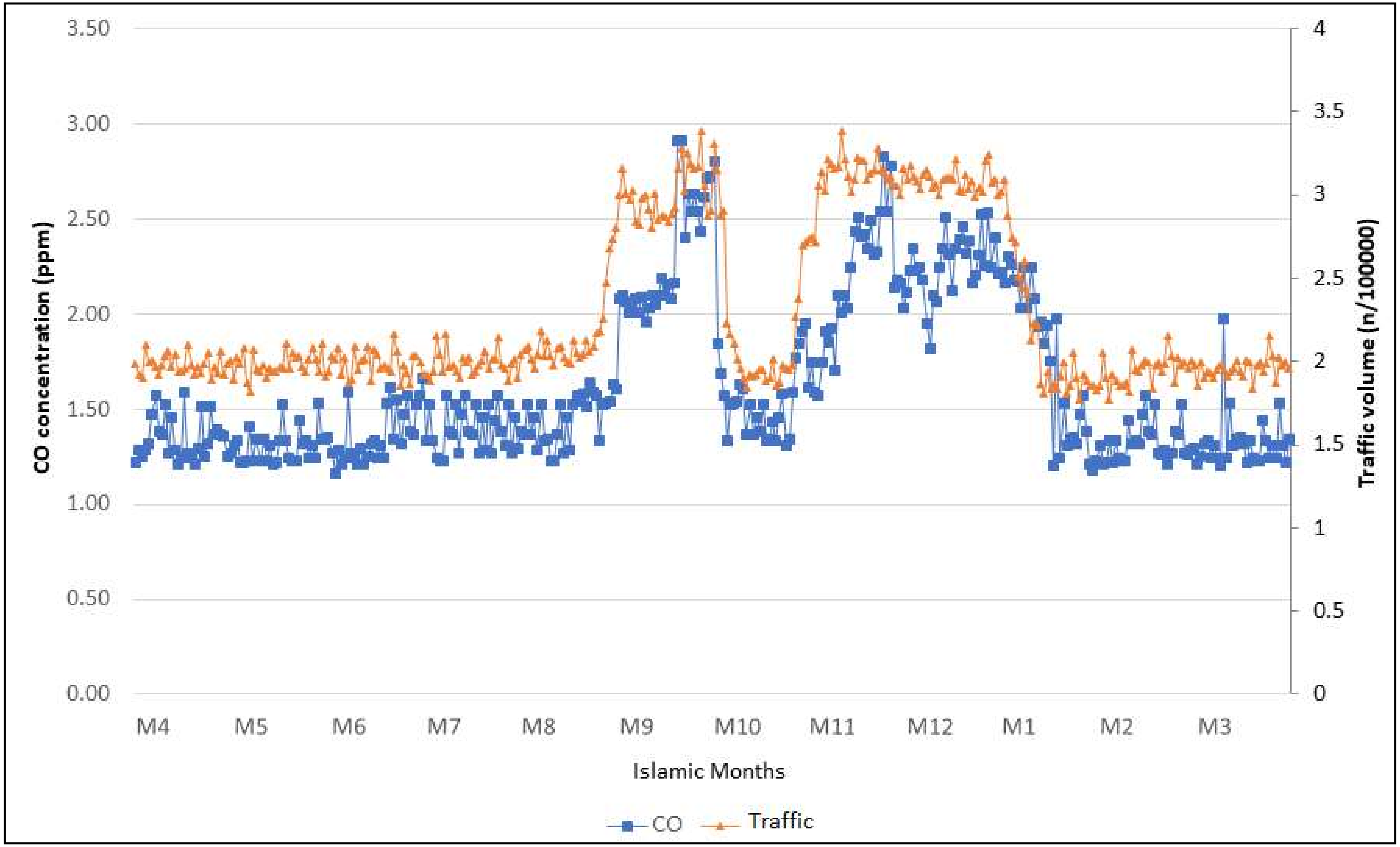


Figure 4-24: Time series plot to compare CO concentration and traffic volume during one year

4.5.7. Relation between CO Concentration and Wind Speed During One Year

The Spearman's correlation analysis results presented in Table 4-19 show the relationship between CO concentration and wind speed during one Islamic calendar year. There was a negative relationship between CO concentration and wind speed, especially during M9, M11, M12 and M1 (months of religious importance except M1). This relationship was demonstrated in Figure 4-25, where wind speed is represented by the small arrows in different directions, and the red colour gradient represents CO concentrations. It can be noticed that lower wind speeds correspond to higher CO concentrations, while higher wind speeds correspond to lower CO concentrations due to the scattering ability of contaminants, which increases with high speed. This is mainly because, on a windy day, CO molecules are swept away at a faster rate than on a calm day.

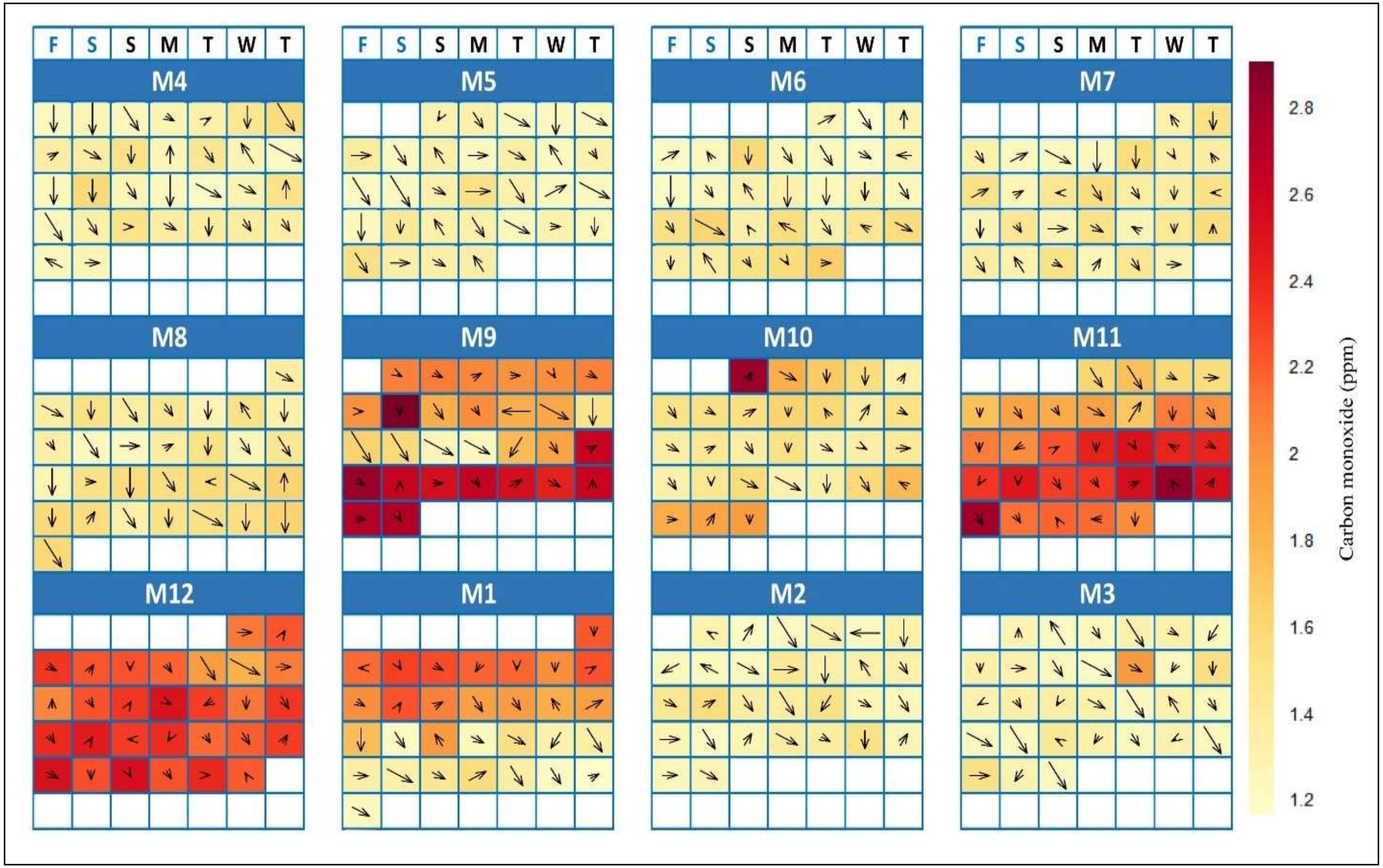


Figure 4-25: Calendar plot for the wind speed with respect to CO concentrations

4.6. Discussion

4.6.1. Summary of the Research Findings

One of the specific objectives of this study was to identify the hot spots of BaP concentrations in Madinah and compare them with the background site (objective 1). The hot spot locations for BaP exposure were the areas associated with high traffic volume (Downtown, Quba and Awali). However, the areas away from traffic or experiencing low traffic volume (Sultana and Al Aziziyah) had less chance of exposure to BaP. With regard to objective 2, which is to identify the major variables (i.e. traffic density, religious events, meteorological variations) that could affect BaP concentrations during one Islamic calendar year, the study reports that a number of factors affected the concentrations of ambient BaP. These factors were: religious events, traffic volume, and meteorological conditions (windspeed and relative humidity). It was found that the holy months, Ramadan (M9), Dhu al-Qadah (M11) and Dhu al-Ḥijjah (M12) were associated with higher concentrations of individual BaP, ambient BaP and CO, which all have a direct relation with traffic volume. In addition, the results did not find a relation between the concentration of BaP and meteorological conditions, but there was a negative relation between wind speed and CO concentration.

4.6.2. Hot spots of BaP Contamination in Madinah

The regions that surround or are near the prophet's mosque are characterized by heavy traffic, which emit high amounts of air pollutants, especially organic compounds related to internal combustion engines (Hameed *et al.*, 2015). In the present study, BaP median concentrations significantly differed between traffic congestion locations and control locations, and higher concentrations shifted towards traffic congestion locations ($P<0.05$). This indicates that (BaP) is mainly related to traffic emission and other combustion activities. BaP is a conventional traffic marker (Ning *et al.*, 2018). PAHs arise from combustion sources and the evaporative of petroleum derived compounds, gasoline vehicles, and industrial sources, and are the main contributor of organic compounds in the air, leading to increased exposure for all in Al Madinah to PAH compounds.

These results agree with those of Alghamdi *et al.* (2015b), who compared ambient PAH conditions in regions with high and low traffic in Jeddah City, Saudi Arabia. The difference between this study and the present one is that Alghamdi *et al.* (2015d) investigated pyrene, while the current study investigated BaP. The results were in agreement as both studies found that locations associated with high volume traffic have high ambient PAH concentrations compared to districts with low traffic volumes.

Another way in which the hot spots of PAH contamination in Madinah were determined was by comparing BaP levels in individuals (street janitors) in different locations in the city. The results showed that street janitors working in areas associated with high traffic volumes had higher BaP concentration in their breath compared to those working in locations associated with low traffic volume. The individual BaP concentration in areas associated with high traffic volumes was found to be approximately 16 times higher than the control locations. The individual BaP concentrations in areas with low and high traffic ranged between 0.12–0.57 ng/m^3 and 0.35–0.37 ng/m^3, respectively, indicating that janitors working in areas associated with high traffic are more exposed to BaP compared to those working in areas associated with low traffic. Therefore, according to these results, the areas with traffic such as Downtown, Quba and Awali are hot spots for PAH contamination.

These results agree with those of Fan *et al.* (2012b), who studied the exposure of children to PAHs as a result of being exposed to traffic-related polluted air in Guangzhou, China. While the current study investigated air inhaled by these participants for BaP concertation, Fan *et al.* (2012b) investigated 2-hydroxynaphthalen, 2-hydroxyfluorene, 3- and 9-hydroxyphenanthrene, and 1-hydroxypyrene, all of which are biomarkers of PAH exposure. The measured BaP concentrations were compatible with the measurements found in Jeddah (0.09–0.39 ng/m^3) (Alghamdi *et al.*, 2015d) Spain (0.29 ng/m^3) (Callén *et al.*, 2010), Kuwait (0.25ng/m^3) (Beg *et al.*, 2009). On the other hand, the results in the present study were significantly lower than those measured in Assuit, Egypt (49.39 ng/m^3) (Abdallah and Atia, 2014), Giza, Egypt (159.55 ng/m^3) (Hassan and Khoder, 2012), Iran (18.71 ng/m^3) (Halek *et al.*, 2010), Agra, India (28.02 ng/m^3) (Masih *et al.*, 2012), and northern China (27.2 ng/m^3) (Xia *et al.*, 2013). Moreover, El-Mubarak *et al.* (2015) reported extremely high concentrations of individual PAH congeners of up to 1003 ng/m^3 with an average concentration of BaP of 400 ng/m3 in Riyad, Saudi Arabia, ~3 order of magnitude larger than those recorded in this study. Generally, the major emission sources of PAHs are thought to be traffic-related activity, which differs from day to day and one month to another. Moreover, these variations may be attributed to the impact of temperature, and methodology (sampling and analysis technique). Temperature has a potential impact on PAH compounds, tending towards high vapour. In Fan *et al.* (2012b), it was found that the child participants living in areas with high traffic had a higher concentration of 2-hydroxynaphthalen, 2-hydroxyfluorene, 3- and 9-hydroxyphenanthrene, and 1-hydroxypyrene in their urine, compared to participants in low traffic areas. This is a clear indication that indeed high traffic areas are hot spots for PAH contamination and people living or working in these areas have a high chance of being exposed.

4.6.3. Comparison of BaP Concentration by Measuring Method

BaP concentration in Madinah was measured using two methods: ambient air concentration, and individual air concentration. This was done to determine the association between ambient air BaP concentration and exposure of people to BaP. The results of this study indicated that the ambient BaP concentration and individual BaP concentration are strongly positively correlated (rs= 0.79). This relationship indicates that the higher the ambient BaP concentration, the higher the chance of people being exposed to BaP, and, therefore, the chances of contracting cancer and respiratory complications as result of exposure to BaP also increases. This means that people living or working in areas with or near high traffic areas in Madinah city are at greater risk, as BaP is one of the most critical PAHs produced as a result of incomplete combustion (Alshaarawy *et al.* (2013) Yang *et al.* (2014). It is often found in coal, tar, fossil fuels, and many types of food such as grilled meats, as well as in tobacco smoke. It is formed when a benzene ring is fused to pyrene. The diol epoxide metabolites of this PAH react with and bind to the DNA, resulting in gene mutation which can lead to lung cancer in the long run. Additionally, it also inhibits lymphocytes (white blood cells) from differentiating into microphases, which are the body's first line of defence in fighting diseases and infections. Exposure to other PAHs is also associated with carcinogenic and mutagenic properties (Tang, 2008; Haritash and Kaushik, 2009; Dvorská *et al.*, 2012).

According to Unwin *et al.* (2006), people are exposed to PAHs via the lungs (breathing), cutaneous routes of absorption, and through ingestion. Additionally, exposure to PAHs by the general population may occur through breathing in vehicular exhausts, smoke from open fireplaces, indoor air saturated with cigarette smoke, and eating food with PAHs (ACGIH, 2005b; CRCE, 2008). People may also be exposed to PAHs at their work places, such as mechanics, drivers and street vendors who breathe in exhausts fumes, or those involved in mining, metalworking and oil refining (See *et al.*, 2006).

BaP or some PAHs in the air concentrations are used as indication of PAH exposure (Fiala et al., 2001). The present study confirms that street janitors may be exposed to BaP at a higher level in traffic congestion locations. The European Union has set a target value of 1ng/m^3 of BaP taken as a representative of PAH mixture and the UK has set air quality objective of 0.25ng/m^3 (Alghamdi *et al.*, 2015d). The results in the present study were almost on the border of the EU threshold and UK objective, indicating that these janitors may be at risk.

4.6.4. Comparison of BaP Emission Rates and CO Concentration

From the results, there is a positive relationship between ambient carbon monoxide and BaP concentrations (Table 4-19). Carbon monoxide concentrations were higher during the holy months and relatively low during the other months (Figure 4-17). When the results of the monthly concentrations of carbon monoxide and BaP were compared, the concentrations of ambient carbon monoxide, ambient BaP and individual BaP were higher in holy months than other months.

As we previously mentioned, Al Madinah and Makkah are the busiest cities in Saudi Arabia, particularly during holy months. Generally, the positive trend of CO concentrations is an indicator of increasing road traffic (volume) (Simpson *et al.*, 2014). Simpson *et al.* (2014) reported that the daily median CO concentrations in the period 1997–2012 in Makkah was 0.98 mg/m^3, and this agrees with the results of the current study.

Also in the present study, median concentrations of CO ranged between 1.5–2.56 mg/m^3, with highest medians in M9 (2.35 mg/m^3) and M12 (2.56 mg/m^3), but this was below the Presidency of Meteorology and Environment (PME) standards (10mg/m^3 for 8 h and 40 mg/m^3 for 1h) (PME 2012). CO is more influenced by local sources. Typically, urban and background monitors describe concentrations which are not directly affected by local sources (busy roads). The primary pollutants (e.g. CO) from traffic will be highest near busy roads, while the secondary pollutants (e.g. O_3) will be lowest near roads.

However, air pollutants vary over hours and days depending on the temporal pattern of sources and meteorological factors. CO concentration is mainly related to busy roads and other anthropogenic activities, and significantly increased during holy months with heavy road traffic. In fact, the change in daily human activity during holy months significantly affected CO and BaP records and the data showed that both pollutants were highly correlated, suggesting they had a common source. The highest traffic volume was found in locations near the holy mosque, which was more impacted by traffic emissions.

Butenhoff *et al.* (2015) found CO levels fluctuated between 12–17 mg/m^3 in the month of Ramadan and averaged 12.6 mg/m^3 in Hajj. There were significantly different CO concentrations in the holy months, and the higher concentrations shifted towards the holy months and exceeding the PME standards. Moreover, CO was found to increase in Makkah during Hajj (Al-Jeelani, 2009), mostly due to increased traffic (Al-Jeelani, 2009).

Katoshevski *et al.* (2011) explain that, aside from CO, vehicles, especially diesel-powered ones, produce PAHs. PAHs are produced alongside CO, and the more the exhaust fumes produced, the more CO is produced, and the more the BaP is produced. Additionally, the greater the traffic volume, the more BaP is emitted and the more CO is emitted into the atmosphere. Because of this, there is a positive relationship between traffic volume and CO concentration, and so the larger the traffic volume, the more the vehicular emissions, and hence the more the carbon monoxide produced. Since holy months such as Ramadan (M9), Dhu al-Qadah (M11), and Dhu al-Ḥijjah (M12) are associated with high traffic volumes as result of many pilgrims travelling to, from and within Madinah, ambient air is likely to be highly polluted with CO and BaP.

4.6.5. Relationship between BaP Concentration and Traffic Volume

Traffic volume is a most important factor that explains the variation in the concentration of BaP in Madinah, and the results indicate that both are directly proportional to each other. From Table 4-10 and Figure 4-13, it can be observed that the traffic volume during the holy months (regardless of the type of vehicle) was higher compared to other months. As a result, the concentration of BaP, both ambient and individual, spiked during these months, and are a clear indicator that areas associated with high traffic volumes are more contaminated with carbon monoxide and BaP, compared to areas associated with low traffic. For this reason, Downtown, Quba and Awali are more contaminated than Sultana and Al-Aziziyah.

These results agree with Alghamdi *et al.* (2015b) and Fan *et al.* (2012a), whose studies were carried out in Jeddah, Saudi Arabia, and Guanzhou, China respectively. These studies found that the regions associated with high volume traffic had high ambient PAH concentrations compared to regions with low traffic volumes, with Fan *et al.* (2012a) finding that participants in high traffic areas were exposed to more PAHs compared to participants in low traffic areas. The reason for the positive relationship between carbon monoxide and BaP contamination and traffic volume is because these contaminants are amongst the major components of vehicular exhausts. According to Katoshevski *et al.* (2011), the key pollutants associated with vehicular exhausts are carbon monoxide, benzene, hydrocarbons (HCs), particulate, oxides of nitrogen and carbon dioxide. The WHO (2005) explains that combustion of fossil fuels by vehicles is associated with the emission of harmful substances often associated with volatile organic compounds, pro-oxidant properties, and polycyclic aromatic hydrocarbons (PAHs).

4.6.6. Religious Factors that Affecting BaP Concentration in Madinah

Another objective of the study was to identify variables that affect ambient concentration of BaP as a form of PAH (objective #2). From the results, it was observed that in regions with high traffic volumes (Downtown, Quba and Awali), the ambient BaP concentration fluctuated throughout the year. It varies from month to other, with highest concentrations achieved during the holy months and lowest during other months (Figure 4-15). Median BaP concentrations were 0.59 ng/m^3 in Ramadan (M9), 0.63 in Dhu al-Qadah (M11) and 0.63 ng/m^3 in Dhu al-Ḥijjah (M12), with concentrations ranging between 0.36–0.43 ng/m^3 in the other months (Table 4-14). In the control regions (comparison sites) such as Sultana and Al Aziziyah, the ambient concentration of BaP was constant throughout the year (Table 4-15). This concentration did not depend on the religious status (holiness) of the month. The median ambient BaP concentration during these months is ~0.02 ng/m^3 (Table 4-15). This is opposed to the regions with high traffic volumes, where religious importance of the month has a high impact on the ambient BaP concentration since high ambient BaP concentrations are experienced during the holy months.

Similar results were realized when individual BaP concentrations in these places were analyzed. Individuals (street janitors) working in regions with high traffic volumes are exposed to high BaP exposure during the holy months (Figure 4-18). For instance, the median individual Bap concentrations were 0.46 ng/m^3 during Ramadan (M9), 0.47 ng/m^3 during Dhu al-Qadah (M11), 0.47 ng/m^3 during Dhu al-Ḥijjah (M12), and 0.39 ng/m^3 during Muḥarram (M1); however, in the other months, the median individual BaP concentration ranged between 0.32–0.39 ng/m^3 (

Table **4-17**). In the control regions, even though there were slightly higher individual BaP concentrations in holy months than other months, the BaP concentration remained relatively low compared to high traffic regions. For example, the individual median BaP concentration ranged between 0.02–0.03 ng/m^3, with 0.03 ng/m^3 exhibited in holy months and 0.02 ng/m^3 exhibited in other months (Table 4-18).

From the above results, it can be seen that the major factors that affect concentration of ambient and individual BaP in Madinah city are the traffic volume and holiness of the month. Regions with high traffic volumes such as Downtown, Quba and Awali tend to have high ambient and individual BaP concentration compared to regions with low traffic such as Sultana and Al Aziziyah. Also, holy months such as Ramadan (M9), Dhu al-Qadah (M11) and Dhu al-Ḥijjah (M12) are associated with high ambient and individual BaP concentration. These findings agree with past studies and support the fact that traffic volume contributes heavily towards PAHs concentration. When Levy *et al.* (2003) determined the variation of PAHs concentration with traffic, they found PAH concentration were significantly higher in high traffic roads than locations far away from roads.

The high PAH concentration during the holy months is attributed to Madinah city experiencing a large number of visitors (most pilgrims) during the holy months (Brinkhoff, 2015).

Because of the large number of people visiting the city during the holy months, the city becomes overcrowded with traffic, and traffic is one of the major contributors of air pollution (Katoshevski *et al.* (2011). The key pollutants associated with traffic, according to Katoshevski *et al.* (2011), are carbon monoxide (CO), benzene and hydrocarbons (HCs) and particulates (these are soot particles and fine dust). Additionally, fuel that is mostly used in road transport (fossil fuels) is associated with harmful substances, which in turn are often associated with volatile organic compounds, pro-oxidant properties, and polycyclic aromatic hydrocarbons (PAHs) (Katoshevski *et al.*, 2011). Therefore, since holy months are associated with high traffic volumes as result of many pilgrims travelling to, from and within the Madinah, ambient air is likely to be highly polluted compared to other months, resulting in high BaP concentrations during those months.

The results of this study differ from the results of the Zhang et al. (2013), who carried out a study to understand the effect of Olympics events on the concentration of air pollutants in Beijing, China, during the 2008 Olympics. The study found that levels of air pollutants before and after the Olympics were higher than during the event. The reason for this is that, during the Olympics, control measures to reduce air pollution in Beijing were implemented. The Chinese government used its authority to control air pollution through specific actions during the Olympics period such as mandating an odd/even plate number rule for traffic control, implementing stricter control on vehicles entering Beijing, reducing or stopping production at certain factories surrounding Beijing, not allowing about 70% of government cars to run, temporarily closing some petrol stations, in addition to increasing bus fleet and transit frequency. Before and after the Olympics, no control measures were applied. In Madinah, however, no control measures are being applied before, during and after religious events, resulting in higher BaP levels during months of religious importance.

4.7. Chapter Summary

BaP is one of the most dangerous PAHs, and is associated with cancer, respiratory illnesses and gene mutation (Tang, 2008; Haritash and Kaushik, 2009; Dvorská *et al.*, 2012). BaP exposure detrimentally affects human health, and an individual can be exposed environmentally or occupationally (as a result of their job). The major hot spots for exposure to BaP in Madinah are the areas associated with high traffic volume. Conversely, the areas in which there is less chance of exposure in Madinah are away from traffic, or those which experience low traffic volume. The months in which people are likely to be exposed to high quantities of BaP are the holy months in the Islamic calendar, such as Ramadan (M9), Dhu al-Qadah (M11), and Dhu al-Ḥijjah (M12). During these months, the concentration of ambient BaP is high, and there is a positive relationship between ambient BaP and individual BaP. This means that the higher the concentration of ambient BaP, the higher individual BaP. Therefore, the higher the concentration of ambient BaP, the higher the chance that an individual will be exposed to BaP. The main reason for high BaP concentration in these months is that they are associated with high traffic volume, with many people travelling to attend religious events.

CHAPTER FIVE

Urinary 1-OHP as an Occupational Exposure Biomarker to BaP

Chapter 5: Urinary 1-OHP as an Occupational Exposure Biomarker to BaP

5.1. Introduction

In this chapter, the other two objectives of this study are addressed: objective three, quantify urinary 1-OHP levels as exposure biomarkers of BaP in street janitors; and, objective 4, to explore the association between BaP concentration and the emergence of urinary 1-OHP biomarker in the urine samples collected from street janitors. The chapter presents the results of urinary 1-OHP in the after-work and morning urine samples collected from the participants on the basis of different locations and per month, to correlate the results of ambient and individual BaP and its effect on human health. The chapter also covers the interpretation of the results of 1-OHP through the data of traffic volume, and holy months. Finally, the chapter ends with a discussion and summary of all the results.

5.2. The Results of Urinary 1-OHP Samples

Before discussing the results of the urine sampling, it is important to understand how the urinary samples were collected and tested for 1-OHP Table 5-20. The two urine samples (one in the morning and another in the afternoon) were collected from each of the 20 participants in each of the five locations. The urine samples were collected for 20 days, providing a total of 2,400 urine samples. The participants' age ranged from 25–35, and their weight was between 73–86 kg, height from 156–177 cm, and average BMI 24.3 kg/cm. The urine samples were used to measure 1-OHP as a biomarker of exposure to BaP emitted in air. The BaP compound is metabolized into 1-OHP and excreted in urine. The twice daily collections from the five study locations were made throughout the study year, except for the weekends (2 days/week).

Table 5-20: Time Plan for Collecting Urine Samples (Morning and After Work)

Islamic Calendar	Gregorian calendar	Number of samples per participant					
		Downtown	Quba	Awali	Sultana	Al-Aziziyah	Total
Rabi al-awwal 3/1438	1 Nov–29 Dec 2016	20	20	20	20	20	100
Rabi ath-thani 4/1438	30 Dec 16–28 Jan 17	20	20	20	20	20	100
Jumada al-ula 5/1438	29 Jan–27 Feb 2017	20	20	20	20	20	100
Jumada al-akhirah 6/1438	28 Feb–28 Mar 2017	20	20	20	20	20	100
Rajab 7/1438	29 Mar-26 Apr 2017	20	20	20	20	20	100
Shaban 8/1438	27 Apr–26 May 2017	20	20	20	20	20	100
Ramaḍan 9/1438	27 May–24 Jun 2017	20	20	20	20	20	100
Shawwal 10/1438	25 Jun–23 Jul 2017	20	20	20	20	20	100
Dhu al-Qadah 11/1438	24 Jul–22 Aug 2017	20	20	20	20	20	100
Dhu al-Ḥijjah 12/1438	23 Aug–20 Sep 2017	20	20	20	20	20	100
Muḥarram 1/1439	21 Sep–20 Oct 2017	20	20	20	20	20	100
Ṣafar 2/1439	21 Oct–18 Nov 2017	20	20	20	20	20	100
Total		**240**	**240**	**240**	**240**	**240**	**1200**

5.2.1. After-work Urine Samples

The after-work urine samples were collected from the participants to determine the concentration of 1-OHP compound at the end of the 8 h work shift. The participants were exposed to traffic BaP emitted from vehicles during their work as street janitors.

A. After-Work Urine Samples at Five Locations

To understand the difference in the 1-OHP between traffic and control locations, the differences in the distribution of data collected from the five locations (Table 5-21) were determined using Kruskal-Wallis and Mann-Whitney tests. The results of these tests (Mann Whitney with Bonferroni-corrected p-value <0.01), as represented in Table 5-21, indicate that the distributions of urinary 1-OHP concentrations in traffic areas were statistically different from the distribution of urinary 1-OHP concentrations in control locations denoted by different letters (a, b). This difference was such that the higher 1-OHP concentrations were recorded in traffic areas rather than in control locations. The 1-OHP concentration in the traffic locations ranged from 0.23–0.88 µmol/mol creatinine, while in the control locations they ranged from 0.07–0.29 µmol/mol creatinine. On the other hand, data in all locations were positively skewed except for Al-Aziziyah, where it was approximately symmetrical. The skewness in the data was due to the fact that traffic locations recorded higher 1-OHP concentrations in holy months than in the normal months, as holy months experienced a higher traffic density than normal months. The symmetrical nature of the 1-OHP data collected in Al-Aziziyah was due to the fact that Al-Aziziyah was associated with low traffic along the study year, resulting in generally lower BaP exposure, which in turn resulted in an approximately constant 1-OHP concentration.

Table 5-21: Urinary 1-OHP concentration (µmol/mol creatinine) in the after-work samples at five locations during the study year

Location	Median	Minimum	Maximum	IQR	Skewness	Distribution
Downtown[a]	0.40	0.23	0.79	0.29	0.953	+
Quba[a]	0.39	0.26	0.79	0.23	1.1	++
Awali[a]	0.40	0.26	0.88	0.31	0.883	+
Sultana[b]	0.16	0.08	0.29	0.05	0.734	+
Al Aziziyah[b]	0.15	0.07	0.22	0.04	-0.078	≈0

Locations with different letters (a, b) were significantly different in their data distribution, IQR: Inter Quartile Range, ≈0: approximately symmetric (≤0.5); +: moderate positive skewness (>0.5 and ≤1.0); ++: high positive skewness (>1.0), SES: Standard Error of Skewness = 0.157

To display the distribution of data based on the statistical values in Table 5-21, the box plots in Figure 5-26 show that the median values of 1-OHP in traffic locations were markedly higher than the corresponding values in the control locations. The figure also clarified the greater variation between concentrations in traffic locations compared to control locations by the difference in the length of upper and lower quartiles Q3 and Q1, especially in Awali and Quba. The wide variation in 1-OHP concentration in traffic locations might be attributed to high traffic volume and BaP concentration during the holy months, while the low variation in the control locations might be attributed to the relatively constant traffic volume and BaP concentrations in those locations throughout the year. Traffic locations exhibited both high and low traffic volumes during one Islamic year. The high traffic volumes appeared during the holy months of Ramadan M9, Dhu al-Qadah M11, Dhu al-Ḥijjah M12, and Muḥarram M1, while the low traffic volumes appeared during the remaining normal months. Corresponding to this variation in traffic volume, there was a wide variation in the 1-OHP concentration in traffic location. However, the control locations, chosen for their non-religious significance, exhibited constant traffic distribution throughout the year, resulting in a relatively constant 1-OHP concentration.

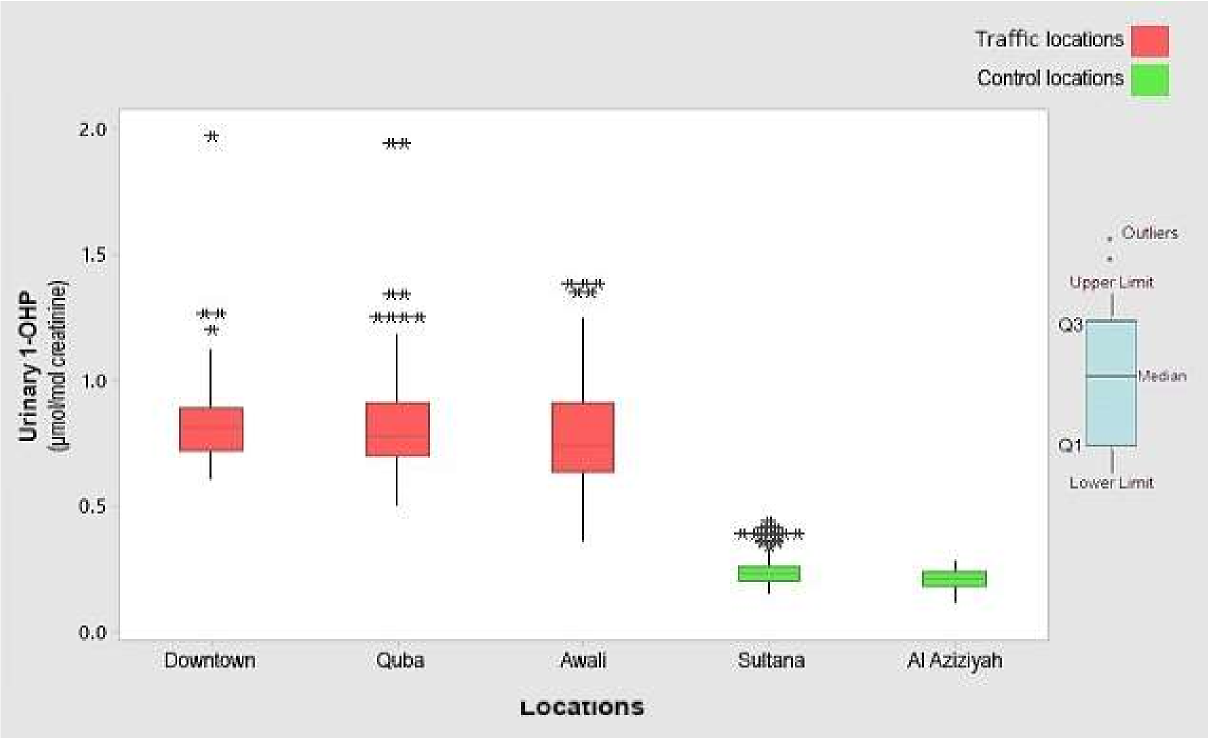


Figure 5-26: Box plot of 1-OHP concentration (µmol/mol creatinine) in after-work urine samples at five locations during the study year

B. After-Work Urine Samples in Traffic Locations During the Study Year

In this section, the results of after-work 1-OHP in urine samples were explained in terms of the months of the study year, as explained in terms of location in the previous section. As represented in Table 5-22, 60 urine samples were collected monthly from the three traffic locations, contributing to a total of 720 samples throughout the study year. The Kruskal-Wallis test with H-value = 395.7 ($p<0.001$) showed a significant difference between 1-OHP data distribution during different months. For more statistical analysis, a Mann-Whitney Test was applied to the 1-OHP data with a Bonferroni-corrected p-value <0.01, resulting in sufficient statistical evidence that data distributions throughout the months of the study year carrying different letters (a, b, c) were markedly different. These results showed that the distribution of 1-OHP concentration in holy months was markedly higher and different from normal months, except for M1, which had intermediate values between those of holy and normal months, as a transition period. Such a difference may be the result of activities which created high vehicle traffic volume in the months of religious importance, such as Ramadan (M9), Dhu al-Qadah (M11), Dhu al-Ḥijjah (M12) and Muḥarram (M1). During the religious months, Madinah tends to receive many pilgrims from all over the world, and, as a result, there is an increase in traffic volume during those months when compared to the normal months. Traffic density was associated with BaP emissions whose atmospheric concentration increases with traffic volume. However, normal months, as earlier discussed, tend to have constant traffic flow throughout the year, resulting in a generally constant 1-OHP concentration.

The difference of 1-OHP concentration in Muḥarram (M1) was brought about by the fact that the first half of that month is considered holy and associated with high traffic, resulting in high BaP and consequently high 1-OHP concentrations. The other half of the month was associated with normal activities and hence the BaP concentration returned back to its normal level. This was generally because it is in the first half of the month that visiting pilgrims start to return home. It was during this month that the concentration of 1-OHP was higher compared to other months, whereas during the other months traffic becomes normal, as most, if not all, of the pilgrims have returned.

Moreover, the data of holy months showed positive skewness (right-tailed distribution), except for M9, in which the data were approximately symmetrical as in normal months. The skewness in the data was a result of the fact that holy months, which are just three and a half months (M9, M11, M12 and first half of M1) of the year, were associated with higher traffic volume, resulting in higher BaP. Exposure to BaP was indicated with 1-OHP concentrations compared to other months, making the median value lower than the mean value.

Table 5-22: Urinary 1-OHP concentration (µmol/mol creatinine) for after-work samples at traffic locations during the study year

Month Number	Month Name	Median	Minimum	Maximum	IQR	Skewness	Distribution
M4	Rabi ath-thani[c]	0.36	0.30	0.44	0.03	0.17	≈0
M5	Jumada al-ula[c]	0.37	0.29	0.41	0.04	-0.14	≈0
M6	Jumada al-akhirah[c]	0.38	0.28	0.45	0.03	0.06	≈0
M7	Rajab[c]	0.36	0.30	0.41	0.04	-0.09	≈0
M8	Shaban[c]	0.37	0.28	0.57	0.05	-0.05	≈0
M9*	Ramaḍan[a]	0.72	0.57	0.78	0.06	0.43	≈0
M10	Shawwal[c]	0.37	0.31	0.57	0.07	0.33	≈0
M11*	Dhu al-Qadah[a]	0.70	0.57	0.78	0.04	0.64	+
M12*	Dhu al-Ḥijjah[a]	0.69	0.58	0.78	0.06	0.63	+
M1*	Muḥarram[b]	0.44	0.33	0.78	0.36	0.81	+
M2	Ṣafar[c]	0.37	0.31	0.41	0.05	-0.04	≈0
M3	Rabi al-awwal[c]	0.35	0.30	0.42	0.05	-0.07	≈0

*Months with different letters (a, b, c) were significantly different in their data distribution, IQR: Inter Quartile Range, *: holy month, ≈0: approximately symmetric (≤0.5); +: moderate positive skewness (>0.5 and ≤1.0), SES: Standard Error of Skewness = 0.309*

The box plot in Figure 5-27 reveals how the large variation between 1-OHP concentrations in holy and normal months is expressed in the upper and lower quartiles Q3 and Q1, as well as the median concentrations. The upper quartile (Q3) and median concentration of 1-OHP in the holy months M9, M11 and M12 exceeded 0.8 µmol/mol creatinine, corresponding to about 0.4 µmol/mol creatinine in the normal months. On the other hand, M1 exhibited a different pattern since it was considered a transition month due to the difference in number of pilgrims associated with different traffic volume and hence BaP exposure and 1-OHP biomarker concentrations. The box plot of M1 showed approximately the same upper quartile (Q3) but a much longer box ranging from less than 0.4 µmol/mol creatinine to 0.8 µmol/mol creatinine.

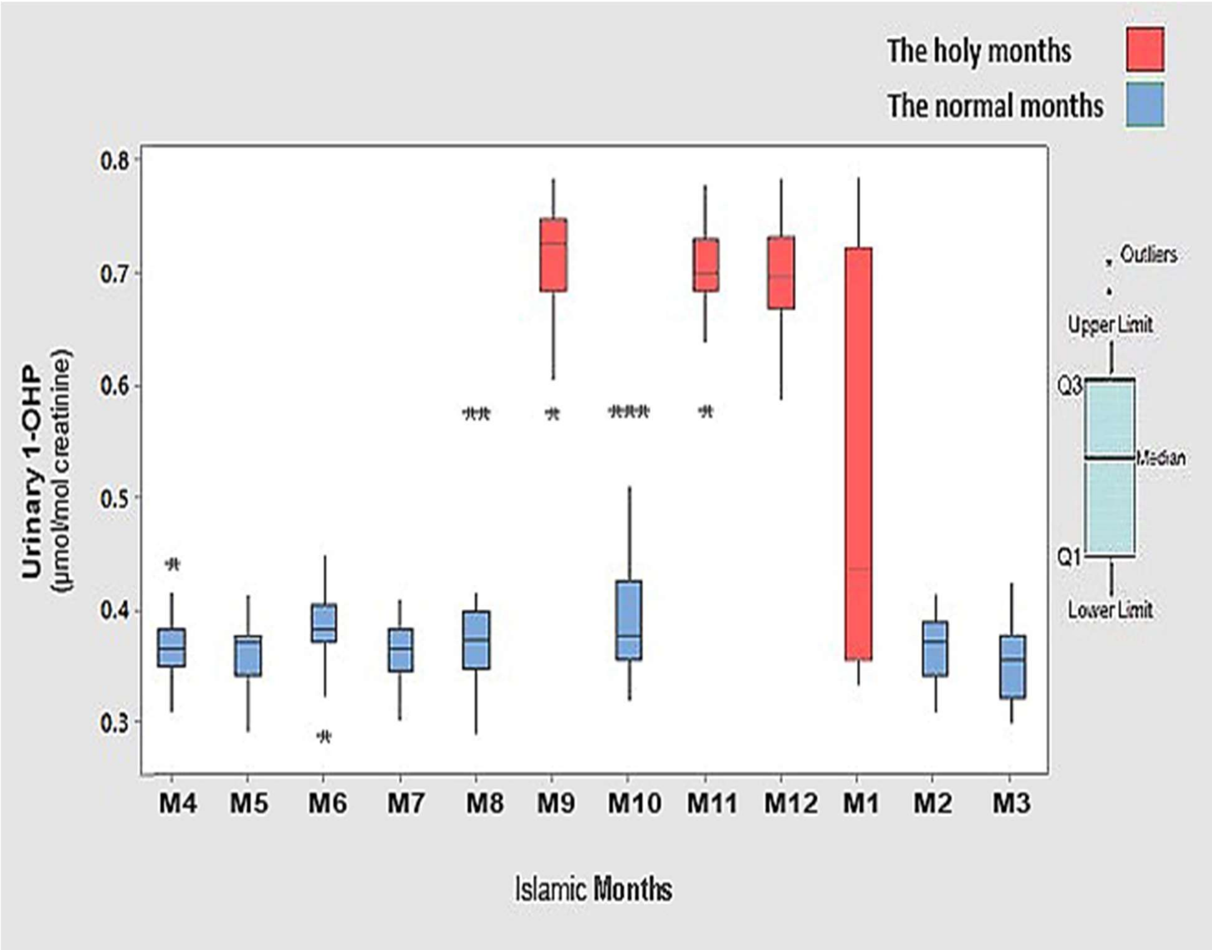


Figure 5-27: Box plot of 1-OHP concentrations (µmol/mol creatinine) in the after-work urine samples in traffic locations during the study year

For more clarification of the obtained results, a time series chart was plotted in Figure 5-28 to demonstrate the distribution of 1-OHP concentrations during months of the study year in both traffic and control locations. Two peaks were obvious in that time series plot: the first peak corresponding to the holy month M9 (Ramadan event), and the other peak corresponding to the holy months including M11, M12 and the first half of M1 (Muslim pilgrim event). The maximum 1-OHP concentration recorded in the traffic locations during the holy months ranged between 0.8–0.9 µmol/mol creatinine, while in the control locations, especially in Sultana, this ranged between 0.2–0.3 µmol/mol creatinine. The normal concentrations recorded in the normal months ranged between 0.4–0.5 µmol/mol creatinine in the traffic locations and between 0.1–0.2 µmol/mol creatinine in the control locations. The plot of the three traffic locations was approximately the same, while, for the control locations, Sultana differed from Al-Aziziyah according to the different nature of both locations, as previously mentioned.

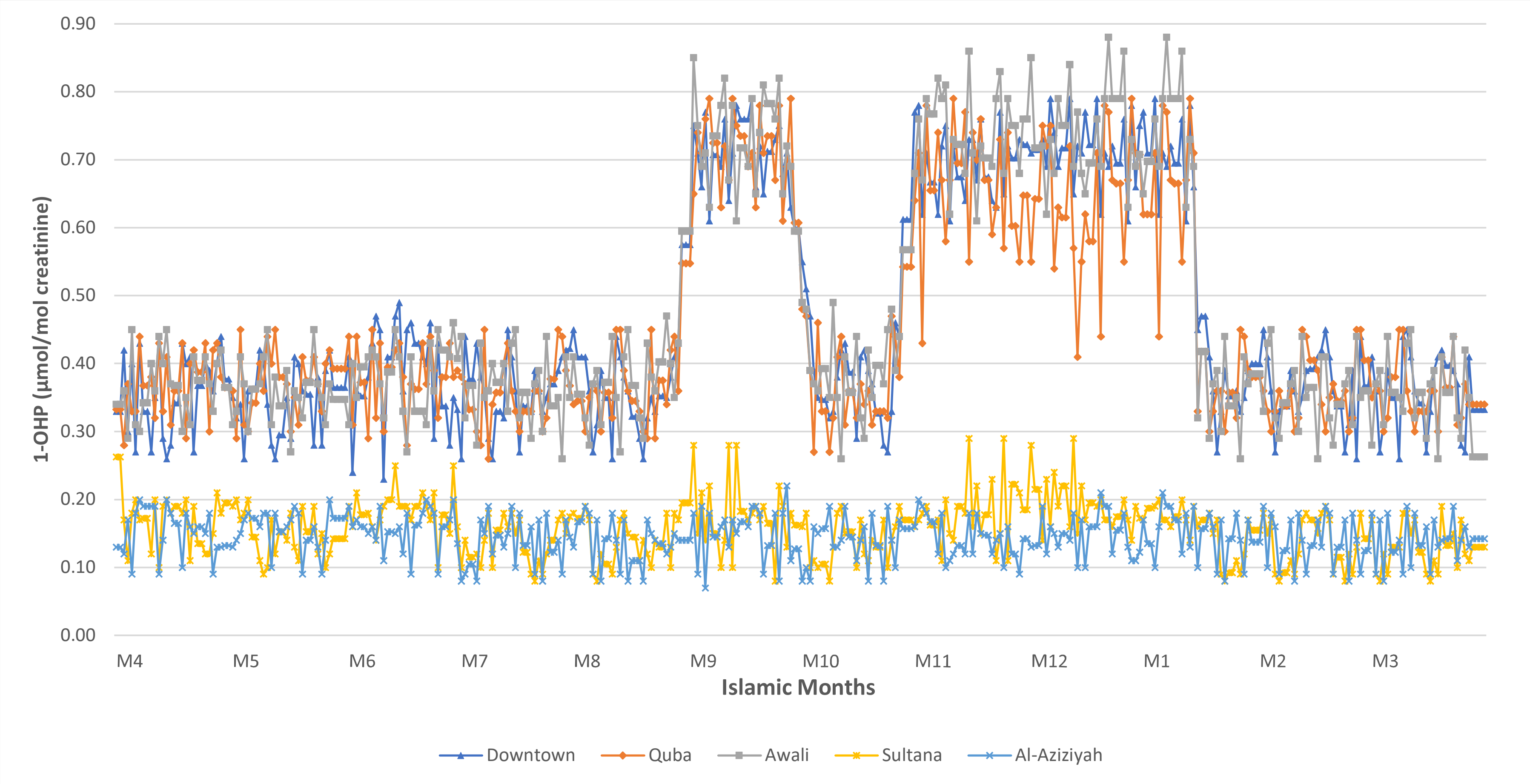


Figure 5-28 : Time series plot of 1-OHP (µmol/mol creatinine) for after-work urine samples at five locations during the study year

C. After-Work Urine Samples in Control Locations during Months of the Study Year

Table 5-23 represented the statistical data of urinary 1-OHP in control locations throughout the months of the study year. 40 urine samples were collected monthly from the two control locations, contributing to a total of 480 samples throughout the study year. The Kruskal-Wallis test with H-value = 53.7 ($p<0.001$) showed that there was no marked difference between the data distribution of 1-OHP concentrations recorded during all months, and hence they are denoted by the same letter (a). On the other hand, there was no skewness data for all months that expressed symmetrical distribution, except for M9, which expressed moderately positive skewness contributing to a moderate distortion of the normal distribution towards the right. The absence of the difference in 1-OHP concentration in all the months in control locations and the absence of skewness show that the 1-OHP concentration in these locations was constant regardless of the time of year. This may be due to the relatively constant traffic volume in those areas. As discussed earlier, control locations have no religious importance in Madinah, so, as a result, traffic volume tends to remain unchanged throughout the year. Because of this, relatively constant 1-OHP concentration values were recorded during the study period of the 12 months of the Islamic year.

Table 5-23: Urinary 1-OHP concentration (μmol/mol creatinine) for after-work samples at control locations during the study year

Month Number	Month Name	Median	Minimum	Maximum	IQR	Skewness	Distribution
M4	Rabi ath-thani[a]	0.16	0.10	0.20	0.04	-0.274	≈0
M5	Jumada al-ula[a]	0.16	0.12	0.19	0.02	-0.163	≈0
M6	Jumada al-akhirah[a]	0.18	0.10	0.23	0.03	-0.135	≈0
M7	Rajab[a]	0.14	0.09	0.19	0.04	0.273	≈0
M8	Shaban[a]	0.13	0.09	0.19	0.04	0.054	≈0
M9*	Ramaḍan[a]	0.16	0.11	0.23	0.04	0.612	+
M10	Shawwal[a]	0.14	0.09	0.18	0.03	0.050	≈0
M11*	Dhu al-Qadah[a]	0.16	0.13	0.24	0.02	0.178	≈0
M12*	Dhu al-Ḥijjah[a]	0.17	0.13	0.24	0.04	-0.001	≈0
M1*	Muḥarram[a]	0.16	0.08	0.19	0.04	0.236	≈0
M2	Ṣafar[a]	0.14	0.09	0.19	0.03	-0.058	≈0
M3	Rabi al-awwal[a]	0.14	0.09	0.19	0.02	-0.245	≈0

IQR is the inter quartile range, ≈0: approximately symmetric (≤0.5); +: moderate positive skewness (>0.5 and ≤1.0), SES: Standard Error of Skewness = 0.374

5.2.2. The Results of Morning Urinary 1-OHP Samples

The morning urine samples were collected from the participants to measure the concentration of 1-OHP compound before their work shift. The participants were exposed to BaP emitted from traffic vehicles during their work as street janitors, with 1-OHP produced by the metabolic processes in the human body during sleep. The main purpose of collecting both morning and afternoon urine samples and testing them for 1-OHP was to determine the best time to gather accurate results with regards to BaP exposure.

A. Morning Urinary 1-OHP Samples at Five Locations

One urine sample was collected daily in the early morning from participants in the five locations. A total of 240 samples/location/year were collected with 114 days missing due to weekends (2 days/week), contributing to a total of 1,200 samples from the five locations. The median concentrations in traffic locations ranged from 0.74–0.81 μmol/mol creatinine while the median concentration in control locations ranged from 0.21–0.23 μmol/mol creatinine.

The statistical analysis using the Kruskal-Wallis test with H-value = 871.0 ($p<0.01$) showed a significant difference between the data distributions of the morning 1-OHP concentrations, and was confirmed by the Mann-Whitney Test with Bonferroni-corrected p-value to the data distributions in traffic and control locations, which indicated a significant difference between locations denoted by different letters (a, b). This means that the urinary 1-OHP concentrations in traffic areas were generally different from the urinary 1-OHP concentrations in control locations. This difference was such that the concentration of morning urinary 1-OHP was higher in locations with high traffic volumes than in locations which experience low traffic.

In fact, the reason for this may be attributed to the high traffic volumes experienced by the traffic locations compared to the control locations throughout the year, as they are associated with high ambient BaP concentrations which increase the exposure chances of people living in those areas. The exposure was confirmed by the presence of 1-OHP in the urine samples of exposed people compared with participants in the control locations, which exhibited relatively low and constant traffic throughout the year, and hence had lower levels of ambient BaP and 1-OHP as a biomarker.

On the other hand, skewness showed that the data distribution of all locations was positively distorted except for Al-Aziziyah, which was approximately normally distributed, as shown in Table 5-24. The reason for the skewness in data was that the locations exhibited both high and low traffic volumes in one Islamic year. They showed high traffic volumes during the holy months of Ramadan M9, Dhu al-Qadah M11, Dhu al-Ḥijjah M12 and Muḥarram M1 and low traffic volumes during the normal month. This difference in the amount of traffic lead to the skewness in data. As a control location with no religious importance, Al-Aziziyah tended to exhibit a constant traffic volume throughout the year, resulting in the normality of data collected from this location.

Table 5-24: Urinary 1-OHP (μmol/mol creatinine) concentration for morning urine samples at five locations during the study year

Location	Median	Minimum	Maximum	IQR	Skewness	Distribution
Downtown[a]	0.81	0.60	1.97	0.17	2.1	++
Quba[a]	0.78	0.50	1.94	0.21	1.6	++
Awali[a]	0.74	0.36	1.38	0.27	0.796	+
Sultana[b]	0.23	0.15	0.44	0.05	1.1	++
Al Aziziyah[b]	0.21	0.11	0.29	0.06	0.046	≈0

Locations with different letters (a, b) were significantly different in their data distribution, IQR: Inter Quartile Range, ≈0: approximately symmetric (≤0.5); +: moderate positive skewness (>0.5 and ≤1.0); ++: high positive skewness (>1.0), SES: Standard Error of Skewness = 0.313

In Figure 5-29, the box plots show that the morning urinary 1-OHP concentrations in traffic locations were markedly higher in its medians than in the control locations. Traffic locations expressed higher 1-OHP concentrations represented by the long box plots compared with those of the control locations. The morning urinary 1-OHP in traffic locations had greater variation throughout all quartiles (long quartiles) than control locations. The variations in traffic locations were greater in the upper half of the ordered concentrations (Q3 and Q4) than in the lower quartile (Q1). However, there were many upper outliers (≥ (Q3+1.5 IQR)) in Sultana. The box plots in the control locations were short throughout all quartiles, indicating much less variation than in traffic locations. This means that there was little variation in 1-OHP concentration in the control locations as all the concentrations were located in the lower quarter (Q1).

The wider variation in morning 1-OHP concentration in traffic locations might be the result of high traffic volume and BaP concentration during the holy months. The low traffic volume variation in the control location, on other hand, might be attributed to a relatively constant traffic volume and BaP concentrations in these locations throughout the year.

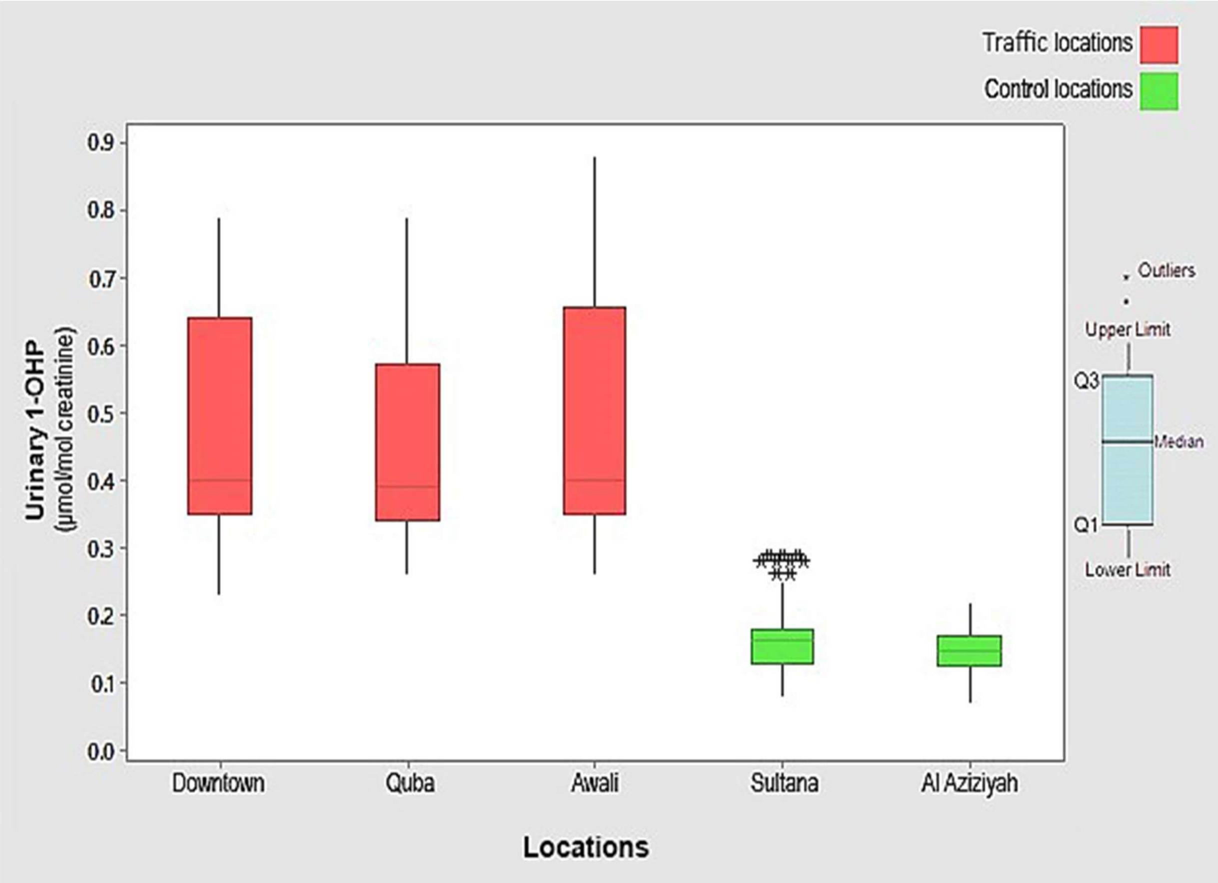


Figure 5-29: Box plot of morning samples concentration of urinary 1-OHP (µmol/mol creatinine) at five locations during the study year

B. Morning Urinary 1-OHP Samples in Traffic Locations during the Months of the Study Year

One urine sample was collected daily in the early morning from participants in the traffic locations. A total of 60 samples per month were collected from the three locations with 114 days missing due to weekends (2 days/week), contributing to a total of 720 samples from the three traffic locations during the study year.

The calendar plot represented in Figure 5-30 shows a general view of the 1-OHP concentrations, with the hot spots recording the highest concentrations. With respect to the colour scale on the right-hand side of the calendar plot, the darker colour gradient points to a higher 1-OHP concentration on this day. From the calendar plot, it can be observed that 1-OHP concentration was generally high during the holy months (M9, M11 and M12) and the first half of M1, when the highest concentration was greater than 0.9 μmol/mol creatinine. This can be attributed to the high traffic volume in those months, resulting in a high ambient BaP concentration whose exposure was expressed in the concentration of urinary 1-OHP. On the contrary, the normal months exhibited lower 1-OHP concentrations, ranging between less than 0.5 and 0.6 μmol/mol creatinine. This is represented by a lighter colour gradient.

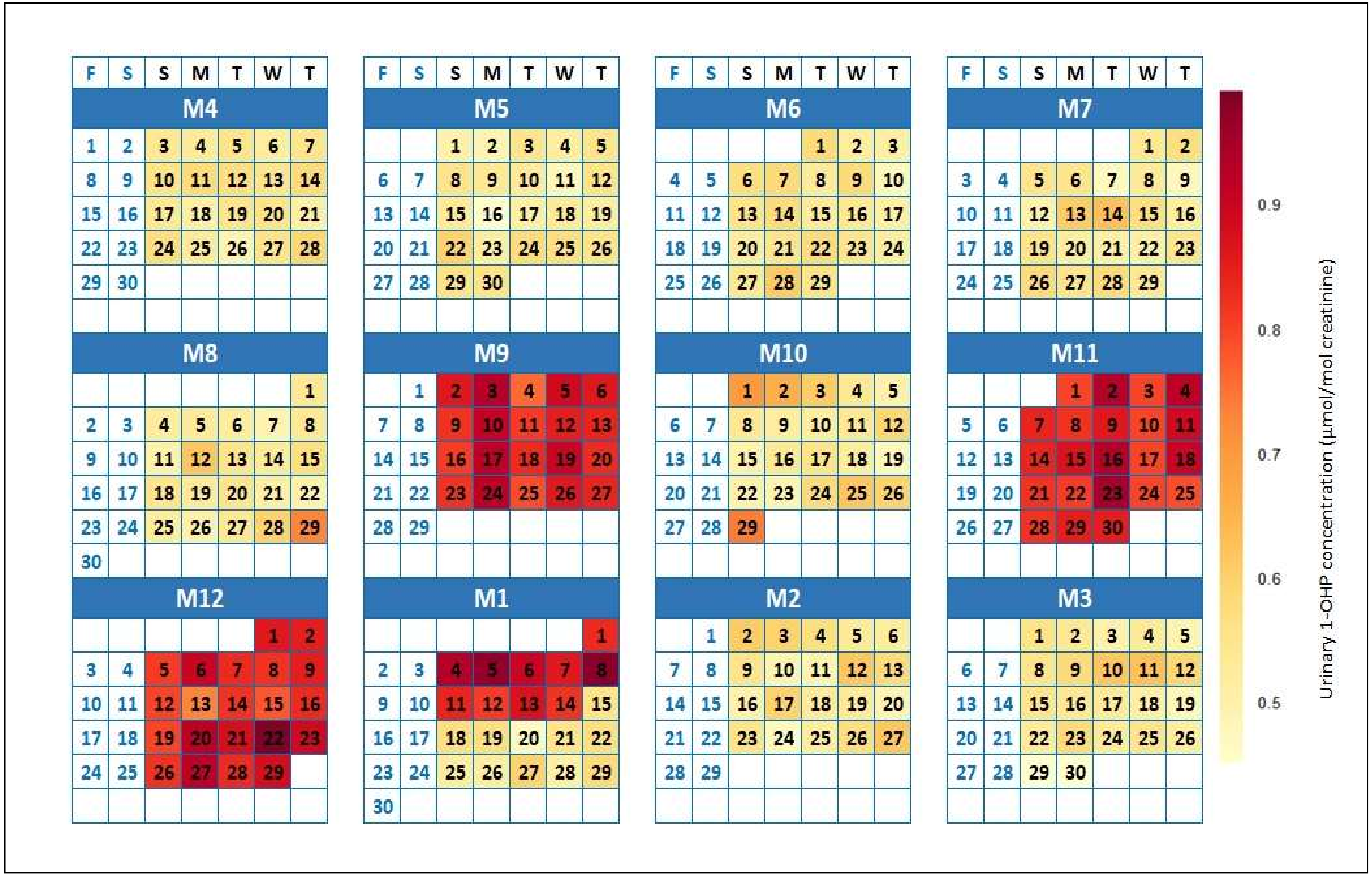


Figure 5-30: Calendar plot of daily urinary 1-OHP concentrations (µmol/mol creatinine) at traffic locations during the study year

Table 5-25 shows that the median concentrations of morning urinary 1-OHP at the traffic locations were markedly higher in M9, M11 and M12 than M1 (0.98, 0.99, 1.01 and 0.82 µmol/mol creatinine, respectively) and those recorded in normal months (0.71–0.74 µmol/mol creatinine). The transition month M1 showed intermediate concentration of urinary 1-OHP between concentrations between that recorded in holy months and normal months due to its unique nature as a transition period.

After testing the normality of morning 1-OHP data using a Shapiro-Wilk Normality Test ($p<0.05$), it was found to be violated, and the Kruskal-Wallis Test with H-value = 363.2 ($p<0.001$) was used to test for the differences between distributions. Marked differences were found and as a result ten Mann-Whitney tests with Bonferroni correction p-value <0.01 were carried out. Months denoted by different letters (a, b, c) were markedly different in the morning 1-OHP data distribution. The difference was such that the holy months of Ramadan (M1), Dhu al-Qadah (M11), Dhu al-Ḥijjah (M12) and Muḥarram (M1) were associated with higher morning 1-OHP concentrations than the normal months. Such a difference in 1-OHP concentration may be due to high BaP concentrations brought about by high vehicle count and traffic volume in holy months compared to normal months.

Table 5-25: Urinary 1-OHP (µmol/mol creatinine) concentration for morning urine samples at traffic locations during the study year

Month Number	Month Name	Median	Minimum	Maximum	IQR	Skewness	Distribution
M4	Rabi ath-thani[c]	0.73	0.61	0.81	0.05	-0.15	≈0
M5	Jumada al-ula[c]	0.72	0.62	0.85	0.08	-0.11	≈0
M6	Jumada al-akhirah[c]	0.71	0.61	0.82	0.06	-0.01	≈0
M7	Rajab[c]	0.73	0.55	0.86	0.04	-0.42	≈0
M8	Shaban[c]	0.71	0.59	0.90	0.09	-0.20	≈0
M9*	Ramaḍan[a]	0.98	0.84	1.13	0.08	-0.12	≈0
M10	Shawwal[c]	0.72	0.60	0.90	0.15	-0.23	≈0
M11*	Dhu al-Qadah[a]	0.99	0.83	1.14	0.06	-0.62	+
M12*	Dhu al-Ḥijjah[a]	1.01	0.83	1.25	0.08	2.30	++
M1*	Muḥarram[b]	0.82	0.61	1.35	0.27	2.20	++
M2	Ṣafar[c]	0.74	0.60	0.84	0.09	-0.55	+
M3	Rabi al-awwal[c]	0.71	0.59	0.85	0.11	-0.53	+

*Months with different letters (a, b, c) were significantly different in their data distribution, IQR is the inter quartile range, *: holy month, ≈0: approximately symmetric (≤0.5); +: moderate positive skewness (>0.5 and ≤1.0); ++: high positive skewness (>1.0), SES: Standard error of skewness = 0.309*

C. Morning Urinary 1-OHP Samples in Control Locations during the Months of the Study Year

Forty morning urine samples were collected monthly from the two locations, contributing to a total of 480 samples during the study year. The median concentrations of morning 1-OHP collected from the control locations, as shown in Table 5-26, ranged between 0.15–0.20 µmol/mol creatinine. According to the Kruskal-Wallis Test with H-value = 29.0 ($p<0.01$), there were marked differences in the data distributions confirmed by applying 66 Mann-Whitney tests with Bonferroni corrected p-value < 0.004. Statistically, months denoted by different letters (a, b, c) were markedly different in the data distribution of morning 1-OHP, which is contrary to all expectations that indicated the stability of 1-OHP concentration in control areas throughout the year, resulting in no statistical difference. However, a closer look at the data in Table 5-26 shows that even though the difference was statistically significant, this difference was not meaningful because there was little variation in the median 1-OHP concentration values, which ranged between 0.16 to 0.20 µmol/mol creatinine. In holy months, they ranged between 0.17–0.2 µmol/mol creatinine and in the normal months they ranged between 0.15–0.18 µmol/mol creatinine. Therefore, morning 1-OHP values tended to be more constant in control locations compared to traffic locations (Table 5-24).

Table 5-25Table 5-26: Urinary 1-OHP concentration (µmol/mol creatinine) for morning urine samples at control locations during the study year

Month no.	Month Name	Median	Minimum	Maximum	IQR	Skewness	Distribution symmetry
M4	Rabi ath-thani[c]	0.17	0.22	0.29	0.03	-0.39	≈0
M5	Jumada al-ula[c]	0.17	0.21	0.25	0.04	-0.23	≈0
M6	Jumada al-akhirah[c]	0.18	0.23	0.29	0.03	-0.17	≈0
M7	Rajab[c]	0.16	0.21	0.27	0.04	0.10	≈0
M8	Shaban[c]	0.16	0.22	0.26	0.03	0.08	≈0
M9*	Ramaḍan[a]	0.20	0.25	0.37	0.05	0.80	+
M10	Shawwal[c]	0.15	0.22	0.26	0.06	0.09	≈0
M11*	Dhu al-Qadah[a]	0.17	0.23	0.35	0.04	0.61	+
M12*	Dhu al-Ḥijjah[a]	0.18	0.24	0.34	0.03	0.45	≈0
M1*	Muḥarram[b]	0.17	0.22	0.35	0.04	0.96	+
M2	Ṣafar[c]	0.16	0.21	0.27	0.03	0.20	≈0
M3	Rabi al-awwal[c]	0.16	0.22	0.27	0.03	-0.04	≈0

*Months with different letters (a, b, c) were significantly different in their data distribution, IQR is the inter quartile range, *: holy month, ≈0: approximately symmetric (≤0.5); +: moderate positive skewness (>0.5 and ≤1.0); ++: high positive skewness (>1.0), SES: Standard error of skewness = 0.374*

Figure 5-31 showed the box plots of urinary 1-OHP concentrations (µmol/mol creatinine) in morning urine samples collected from participants in the control locations. The median, minimum and maximum concentrations in M9, M11 and M12 were similar to one another and markedly higher than normal months, which were also similar to each other. In contrast, M1 was unique in its box plot, showing a wide variation in urinary 1-OHP concentration represented by the very long box; in this regard, the median concentrations resembled the highest values reached in normal months, and the minimum concentrations resembled its minimum values, while the maximum concentrations resemble the median values of holy months. This confirms the unique nature of M1, as discussed earlier, in that the first half of M1 is considered a holy month associated with high traffic while the second is considered a normal month associated with low traffic volume when pilgrims start their journey home.

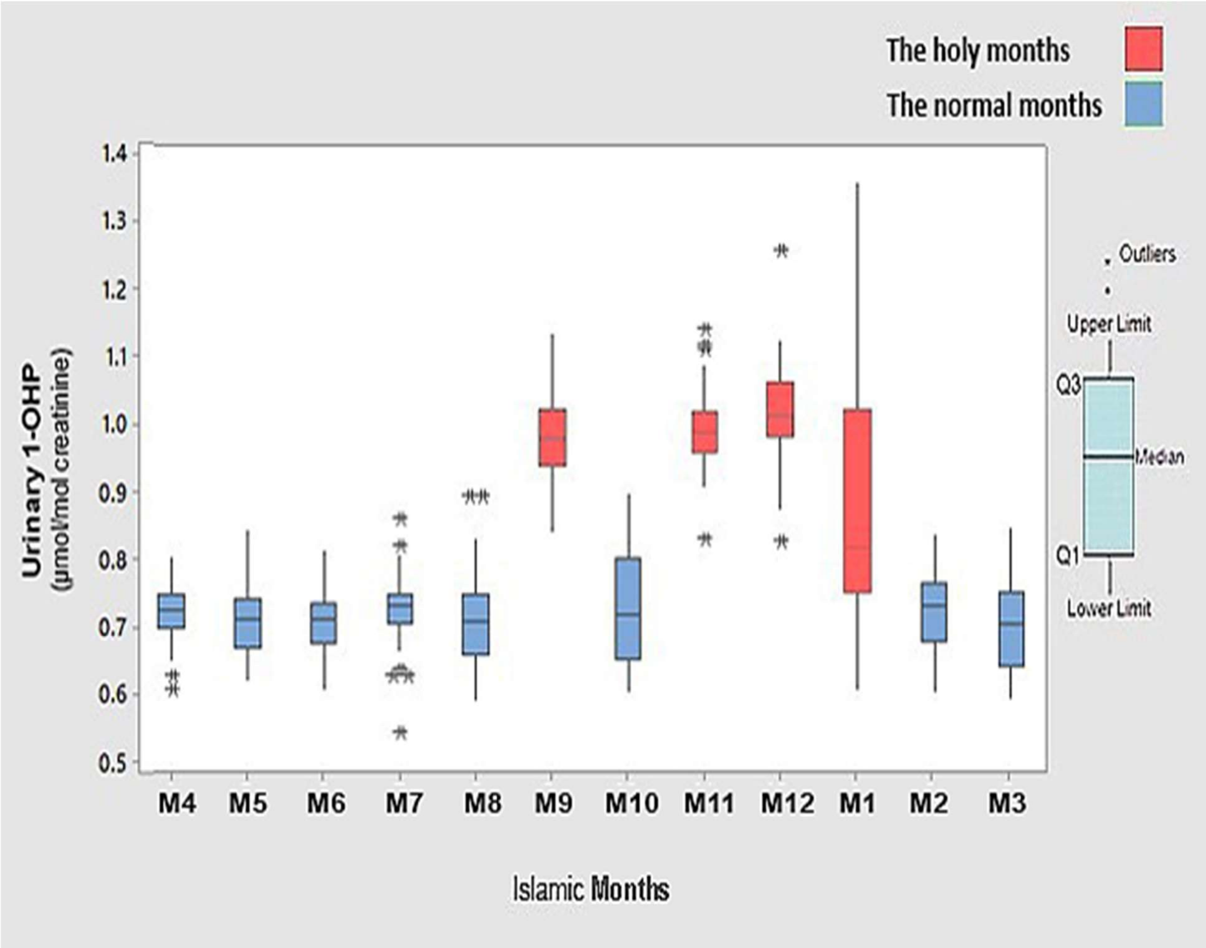


Figure 5-31: Box plot of the morning urinary 1-OHP concentrations (µmol/mol creatinine) from control locations during the study year

Moreover, the time series plot illustrated in Figure 5-32 exhibited two clear peaks from the data of the morning urine 1-OHP samples. The maximum median concentrations recorded in the holy months ranged between 1.1–1.2 µmol/mol creatinine during M9 and 1.1–1.5 µmol/mol creatinine in M11, M12 and the first half of M1. Peaks were detected in all the traffic locations and in the Sultana control location, but not in Al-Aziziyah.

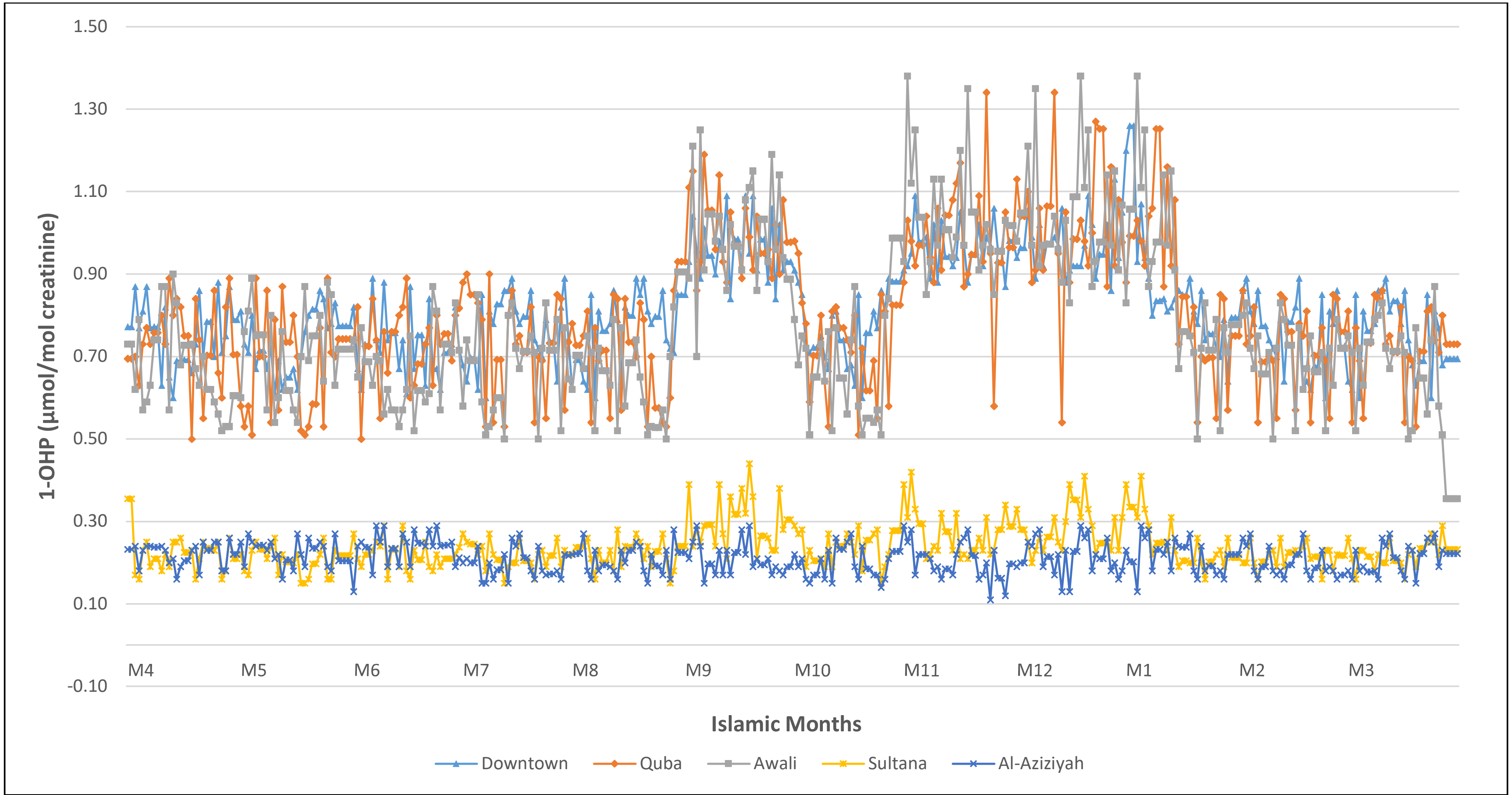


Figure 5-32: Time series plot of 1-OHP (µmol/mol creatinine) for morning urine samples at five locations during the study year

5.3. The Relations between the Findings

This subsection covers the results regarding the relationship between 1-OHP and the various factors (meteorological, BaP, CO, and traffic) that might affect the concentration of urinary 1-OHP. To determine this relationship, a correlation analysis was carried out. Firstly, the relationships determined in this chapter concern the concentration of urinary 1-OHP (morning and after-work) and meteorological conditions, such as temperature, humidity, wind speed and precipitation. Secondly, the relationship between the concentration of urinary 1-OHP (morning and after-work) and BaP (individual and ambient) was also determined, as well as CO and traffic volume. Thirdly, the correlation between 1-OHP concentration and CO concentration was determined. Finally, the correlation between traffic volume and 1-OHP concentration was determined.

5.3.1. Correlations between Air Pollutants and Weather Variables

The results of the correlation analysis are shown in Table 5-27. From the table, a significant positive correlation was observed between the number of vehicles, BaP concentration (both ambient and individual), carbon monoxide concentration, and the concentration of urinary 1-OHP. The results, however, found no relationship between the concentration of urinary 1-OHP and the various weather conditions (temperature, wind speed, precipitation and relative humidity).

Table 5-27: Correlations between different parameters at the five locations in Madinah during the study year

Parameters	Morning Urine	After-Work Urine	Individual BaP	Ambient air BaP	Carbon monoxide	Daily Number of Vehicles	Mean of Temperature	Humidity	Wind Speed	Precipitation
Morning Urine Urinary 1-OHP (μmol/mol creatinine)	1	0.91**	0.89**	0.85**	0.45**	0.86**	0.16ns	-0.14 ns	0.04ns	-0.01ns
After Work Urine Urinary 1-OHP (μmol/mol creatinine)		1	0.78**	0.74**	0.48**	0.81**	0.26ns	-0.22 ns	0.08ns	-0.01ns

***: Correlation is significant (p-value < 0.01, 2-tailed), ns: correlation is not significant (p-value >0.05, 2-tailed), N= 1770*

5.3.2. Comparison between Urinary 1-OHP Concentration in Morning and After-Work Samples

Morning and after-work readings of urinary 1-OHP concentration from the same participant were considered paired data, so a Wilcoxon Signed Ranks Test was used to compare the mean difference between both readings. Table 5-28 shows that the concentrations of morning urinary 1-OHP were markedly higher than the after-work median in the five study locations, according to the Wilcoxon Signed Ranks test (p-value <0.001). The results from the table show that generally morning 1-OHP concentrations were higher than after-work, because it takes 12–24 h for the absorbed BaP to hydrolyze into 1-OHP. It was also observed that 1-OHP concentrations in traffic locations such as Downtown, Quba and Awali were generally higher than 1-OHP in control locations.

Table 5-28: Median urinary 1-OHP concentration (µmol/mol creatinine) in the after-work and morning urine samples at five locations during the study year

Location	After-work urine median	Morning urine Median	Z-value	p-value
Downtown	0.40	0.81	-13.432-[a]	<0.001
Quba	0.39	0.78	-13.406-[a]	<0.001
Awali	0.40	0.74	-13.428-[a]	<0.001
Sultana	0.16	0.23	-12.675-[a]	<0.001
Al-Aziziyah	0.15	0.21	-12.756-[a]	<0.001

Table 5-29 also shows that morning urinary 1-OHP concentrations were higher than after-work concentrations. The higher concentration of 1-OHP in the morning samples confirms that the absorbed BaP due to exposure had been fully hydrolyzed into the urine, thus giving a more accurate measure of BaP exposure than after-work readings. Additionally, the table shows that, regardless of the time when the urine samples were collected, months M6, M8, M9 and M10 generally had higher 1-OHP concentration than other months.

Table 5-29: Median urinary 1-OHP concentration (µmol/mol creatinine) in the after-work and morning urine samples at months of the study year

Months	After-work urine median	Morning urine median	p-value
M1	0.36	0.73	<0.001
M2	0.37	0.72	<0.001
M3	0.38	0.71	<0.001
M4	0.36	0.73	<0.001
M5	0.37	0.71	<0.001
M6	0.72	0.98	<0.001
M7	0.37	0.72	<0.001
M8	0.70	0.99	<0.001
M9	0.69	1.01	<0.001
M10	0.44	0.82	<0.001
M11	0.37	0.74	<0.001
M12	0.35	0.71	<0.001

5.3.3. Comparison between After-Work and Early Morning Urinary 1-OHP

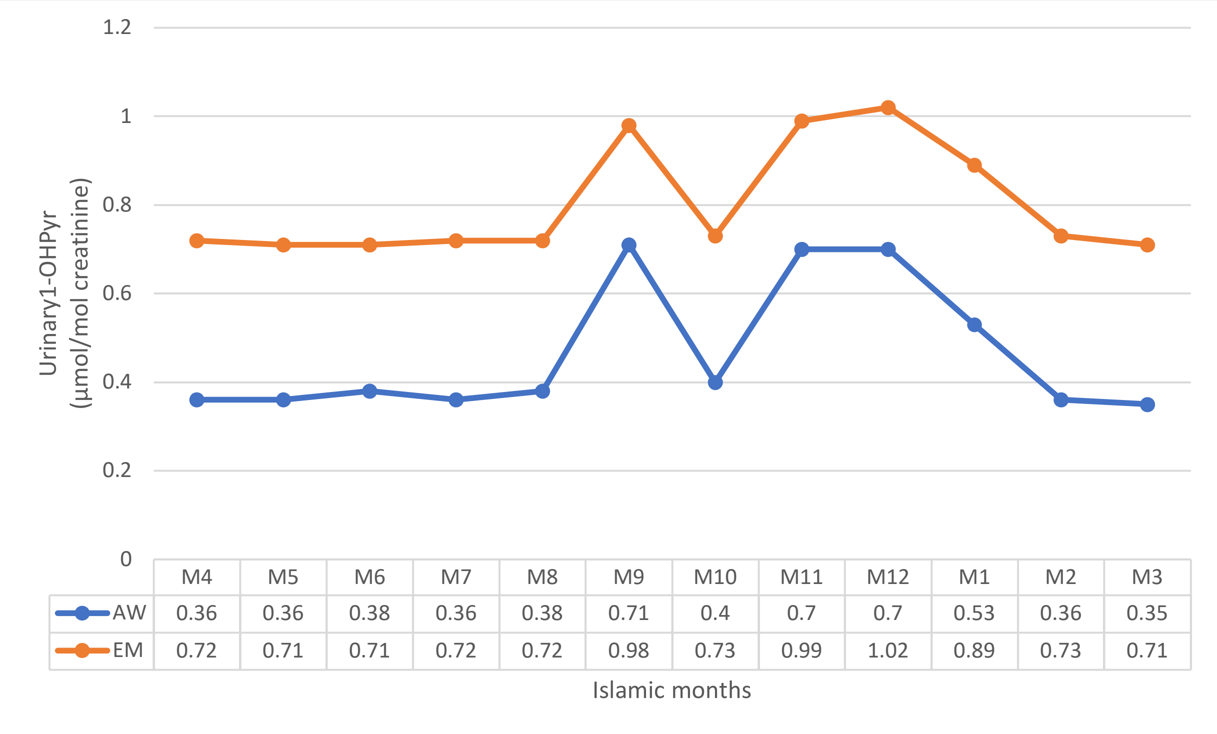


Figure 5-33: Median concentration of after work (AW) and early morning (EM) urinary 1-OHP (µmol/mol creatinine) during the study year

Figure 5-33 compares morning and after-work median 1-OHP concentration (µmol/mol creatinine) in Madinah. It is obvious that the concentration of urinary 1-OHP (µmol/mol creatinine) in the morning urine samples was higher than the after-work samples, and that the two lines are parallel over one year, confirming that 1-OHP was a biomarker for exposure to the air pollutant BaP from the previous working day. The higher concentration of 1-OHP in the morning was due to the fact that the ambient BaP absorbed by the participants had been fully hydrolyzed into the urine, thus giving a more accurate measure of BaP exposure than after-work readings.

5.3.4. The Relation between Urinary 1-OHP and Individual BaP Concentration

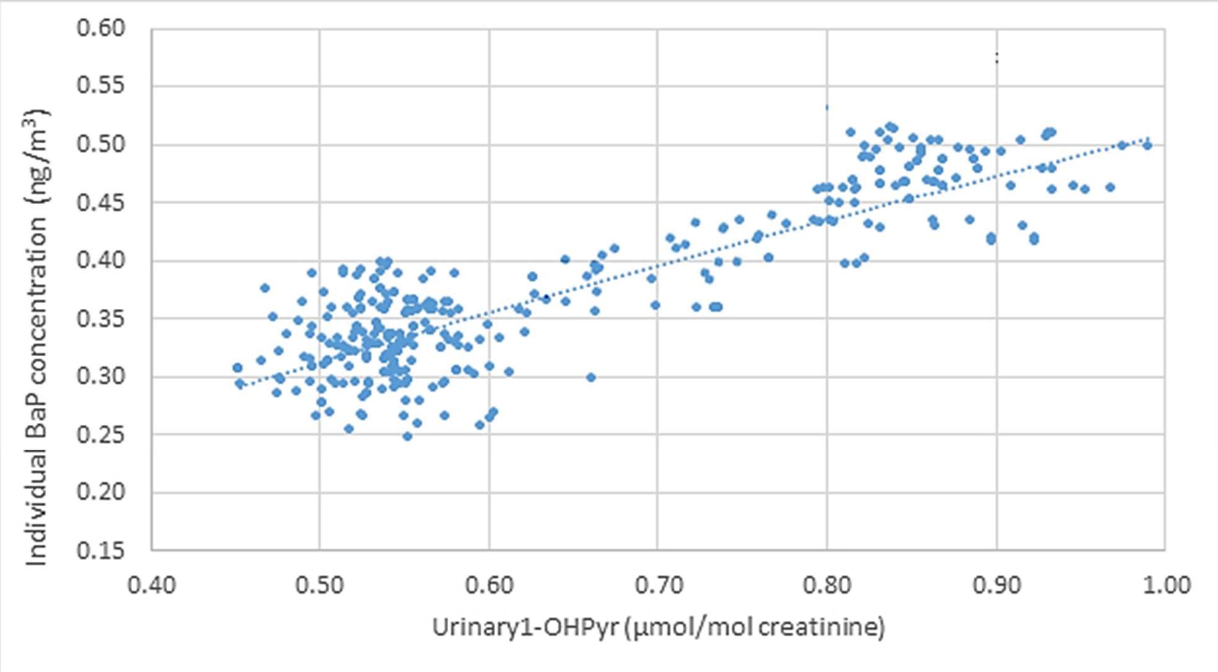


Figure 5-34: Scatter plot of the relation between the concentration of median daily urinary1-OHP and individual BaP with the linear fit line and power curve fit

Figure 5-34 shows the scatter plot of the relation between the median daily urinary 1-OHP concentration and individual BaP concentration with linear and non-linear fit lines. The scatter values show some clustering at certain values of individual BaP, which might be related to the precision of the personal air sampler. The dispersion of values around the fit lines in the scatter plot suggests a positive correlation between both measurements. From the figure, it can be observed that there is a linear positive relationship between daily urinary 1-OHP concentrations and individual BaP concentrations. This positive relationship was also confirmed by Spearman's rank correlation test, which showed a highly significant positive correlation between the mean daily urinary 1-OHP concentration and individual BaP concentration (Spearman's correlation coefficient (r) =0.842, p<0.001, n=1200). The positive association between BaP and 1-OHP confirms that urinary 1-OHP concentration is an accurate measure for exposure to BaP.

5.3.5. The Relation between Ambient BaP, Urinary 1-OHP Concentration and Traffic Volume over One Year

Figure 5-35 is a time series graph comparing 1-OHP concentration, BaP concentration and traffic volume during one Islamic calendar year. From the figure, it can be observed that there was also a positive relationship between these three variables. It was observed that a lower traffic volume corresponds to a lower BaP concentration and a lower 1-OHP concentration. Furthermore, the months of religious importance (M9, M11 and M12) were associated with high 1-OHP concentration, high traffic volume and ambient BaP concentrations. The reason for the sudden increase in traffic volume, BaP and 1-OHP during months M9, M11 and M12 was that these are considered holy months in the Muslim calendar. Figure 5-35 also shows that the holy months exhibited the maximum median values in the three parameters compared with the normal months.

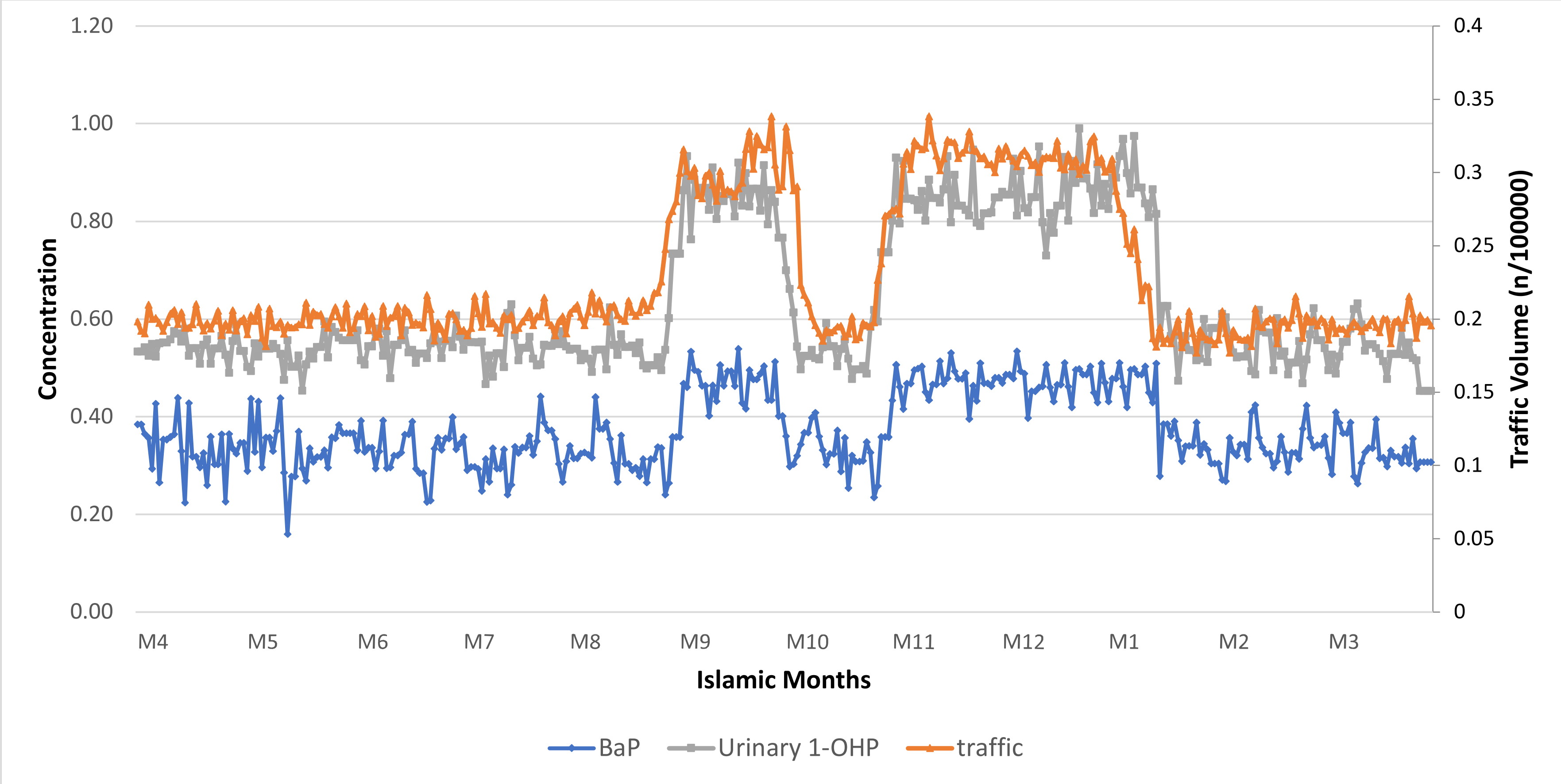


Figure 5-35: Time series plot to compare ambient BaP, urinary 1-OHP concentration and traffic volume during one year

5.4. Discussion

5.4.1. Introduction

In this chapter, the results of the analysis of the urine samples collected from 240 participants for urinary 1-OHP are discussed. Specifically, the chapter compares the concentration of the 1-OHP biomarker in the urine of street janitors in five locations of Madinah, via Downtown, Quba, Awali, Sultana and Al Aziziyah in one Islamic calendar year. The chapter also discusses the extent to which BaP concentration is associated with the concentration of the urinary 1-OHP biomarker, and then compares morning and after-work 1-OHP levels in urine samples.

5.4.2. Summary of the Findings

Generally, the areas associated with high traffic volumes, the workers had higher urinary 1-OHP levels when compared to those in areas associated with low traffic areas (control). The urinary 1-OHP concentrations were higher during the months of Ramaḍan, Dhu al-Qadah and Dhu al-Ḥijjah. However, the urinary 1-OHP concentrations were constant during the normal months. In addition, there was a positive relationship between BaP concentrations (ambient and individual) and 1-OHP concentrations in urine samples, as gradient concentration of BaP was related to gradient 1-OHP concentration.

In addition, the results of the present study indicated that the morning 1-OHP concentrations were higher than after-work 1-OHP concentrations. This is because the appropriate time for collecting urine samples for 1-OHP analysis is the next early morning (pre-shift), as most metabolic reaction processes occur during the night hours, making biomarker compounds more concentrated than any other time of the day.

5.4.3. Comparison of 1-OHP Levels in Street Janitors in Madinah

The results of the present study showed that urinary 1-OHP levels were high after-work and in the morning time in street janitors working in areas associated with high traffic volume during months of Islamic religious. However, urinary 1-OHP levels were consistently low among street janitors working in areas associated low traffic volumes (control areas) and in the normal months. Traffic volume is always associated with 1-OHP levels as it is a major contributor of PAH/ BaP. Airborne BaP correlated well with levels of carcinogenic 4–6 ring PAHs and was an effective marker of exposure for all industries where significant particle-bound PAH levels were found (Jongeneelen, 2014). PAH is oxidized in the human body to form hydroxylated metabolites. Urinary-1-hydroxypyrene (1-OHP) has been used extensively as an exposure biomarker, because it is related to metabolites of other PAH (Keller *et al.*, 2008) and has short biological half-life, representing a recent exposure (Li *et al.*, 2014).

This is not the first research-study to relate BaP concentration with urinary 1-OHP levels, as many studies have been carried out to determine the relationship between the level of exposure to BaP and 1-OHP level. However, it is important to note that most of these studies have not been carried out on street janitors, but on coke oven workers, traffic police, school children, taxi drivers, office workers and bus drivers (Table 2-5). Despite this difference in participants, the results of the present study indicated that street janitors were equally exposed to high levels of BaP. Ifegwu *et al.* (2012) stated that, in some cases, the level of exposure to BaP could be related to occupational and non-occupational settings. In occupational settings, the levels of PAHs were normally elevated and people were more likely to be exposed to higher PAH levels than in non-occupational settings.

For this reason, street janitors, just like coke oven workers, taxi drivers, bus drivers and traffic police, may be exposed to elevated levels of BaP. Jeng *et al.* (2013b) investigated the relationship between 1-OHP levels and exposure to PAHs in 100 coke-oven workers. They found a positive relationship between 1-OHP levels and BaP concentrations. Lin *et al.* (2006) and Wu *et* *al.* (2002) determined the relationship between levels of urinary 1-OHP and occupational exposure to airborne PAHs on coke oven workers. These studies found that the levels of urinary 1-OHP were correlated with occupational exposure to airborne PAHs, such as BaP. 1-OHP is considered an appropriate biomarker for exposure to PAHs because pyrene is rapidly distributed, metabolized and eliminated from the body (Aquilina *et al.*, 2010).

It can be concluded that janitors working in areas associated with high traffic volumes are exposed to high levels of BaP in comparison to exposure levels in street janitors in low traffic areas. In the present study, 1-OHP concentrations were low in comparison with other studies, for example, in Israel (140 ng/g), Germany (110 ng/g) and the US (80 ng/g) (Lutier *et al.*, 2016). This is due to the PAH burden being determined by intake and metabolism/ execretion; intake was relatively low, as it was determined in relation to airborne particulate (solid phase) levels only and not to diet or dermal intake. Generally, inhalation exposure is an important contribution to 1-OHP and 1-OHP concentration in urine increased ~1% in relation to an increase in ambient BAP (Alghamdi *et al.*, 2015b).

Freire *et al.* (2009) estimated that respiratory dose accounted for 5–7% of total absorbed intake of pyrene for children 3–6 years in Canada, living at two sites with concentrations of 27 ng/m^3 and 0.4 ng/m^3, while the remaining dose came from the diet. In the Czech Republic, Freire *et al.* (2009) studied children of 3–6 years exposed to outdoor pyrene concentrations ranging between 0.043–1.5 ng/m^3, and this is compatible with the results in the current study. The authors found that air inhalation was ~4–5% of the observed dose at the high polluted site and the relationships of both ingestion and inhalation doses with 1-OHP in urine were very weak.

In the present study, it is clear that the areas associated with high traffic volumes are more contaminated with BaP in comparison to areas associated with low traffic. For this reason, Downtown, Quba and Awali are more contaminated compared to Sultana and Al-Aziziyah. This finding is in line with Fan *et al.* (2012b) in Guangzhou, China, and Alghamdi *et al.* (2015c) in

Jeddah, Saudi Arabia. These authors found that the workers in areas associated with high traffic were exposed to more PAHs compared to workers in areas associated with low traffic.

Exposure to BaP is linked to a number of health complications. Its diol epoxide metabolites tend to react with and bind to DNA, resulting in gene mutations and eventually causing cancer (Kim *et al.* (2014b). Exposure to BaP is also associated with white blood cell problems, inhibiting some of these cells from differentiating into macrophages (the body's first line defence in fighting infections). Therefore, there is sufficient evidence linking BaP with cancer, and it has been categorized as a group one carcinogen (Kim *et al.*, 2014b).

5.4.4. Monthly Variations of 1-OHP Levels in Madinah

The results of the present study have shown that urinary 1-OHP levels were higher in areas associated with high traffic volume and in the holy months than those in areas associated with low traffic volume and in non-holy months. The holy months (Ramaḍan, Dhu al-Qadah and Dhu al-Ḥijjah) are often associated with high traffic volumes as a result of the religious importance of these months to the Muslim community. BaP levels were found to be high during holy months when compared to other months of the Islamic year. A positive relationship was found between traffic volume and BaP concentration. The results in the present study are in agreement with other studies worldwide.

Leem *et al.* (2005) found that taxi drivers had higher 1-OHP concentrations in their urine. Kamal *et al.* (2016) investigated the effect of traffic-related PAH exposure on traffic police and office workers, finding that traffic police have higher 1-OHP concentrations in their urine than office workers. Chuang (2007) studied levels of concentration of 1-OHP in taxi drivers and office workers, and found that taxi drivers had high 1-OHP concentrations in their urine.

1-OHP is a biomarker for BaP exposure, and a higher level of urinary 1-OHP indicates higher exposure to BaP in the street janitors during the holy months. Moreover, the positive relationship between urinary 1-OHP (an indicator of BaP exposure) and traffic volume is attributed to BaP being a major component of vehicular emissions. The key pollutants of vehicular exhausts are carbon monoxide, benzene, hydrocarbons, particulate, nitrogen oxides, and carbon dioxide (Katoshevski *et al.*, 2011). Since the combustion of fossil fuels by vehicles is associated with emission of BaP into the atmosphere (WHO (2005), the months experiencing high traffic volume may be associated with high 1-OHP levels in the urine samples of the people living or working near these areas, such as the street janitors.

5.4.5. Association between BaP Concentration and 1-OHP Level

The ranking of the correlation coefficients showed that the BaP concentrations and 1-OHP levels of street janitors were positively correlated. This corresponds with other studies that have found positive relationships between urinary 1-OHP levels and BaP concentrations, worldwide. Jeng *et al.* (2013a) found a positive relationship between 1-OHP levels and BaP concentration in 100 coke-oven workers. Kim *et al.* (2000) found that levels of urinary 1-OHP were positively correlated to occupational exposure to airborne PAHs. Liu *et al.* (2007) found positive correlation between levels of 1-OHP and occupational exposure to PAHs in the air on 75 coke-oven workers in Taiwan. Moreover, Wu *et al.* (2010) used Pearson's correlation analysis to determine the relationship between PAH and 1-OHP level on 80 Taiwan coke-oven employees, finding a significant-positive correlation between post-shift levels of urinary 1-OHP and benzene soluble fraction in the air. These positive relationships are explained by Jeng *et al.* (2015) as being due to the fact that urinary 1-OHP is considered a major by-product of pyrene metabolism, which is abundant in BaP. Once BaP is absorbed into the body, the pyrene in it metabolizes to 1-OHP, which is then released from the body via urine, representing more than 90% of the pyrene metabolites. There was a highly significant correlation between location of residence and 1-OHP, suggesting the important concentrations of air exposure (Lee *et al.*, 2011), and this is in line with the higher levels of 1-OHP found during the holy months in the janitors in the current study.

5.4.6. Comparison between Morning and After Work-Urinary 1-OHP Levels

The results showed that morning urinary 1-OHP concentrations were generally higher than after-work concentrations, because 1-OHP has a half-life ranging 18–20 h (Kim *et al.*, 2014b). This means that when an individual is exposed to BaP during work hours, complete metabolism of pyrene takes place during the night. Generally, the most appropriate time for collecting urine samples for 1-OHP analysis is early morning the next day (pre-shift), as most metabolic reaction processes occur during the night hours (Alshaarawy *et al.* (2013). Owing to the kinetics of the absorption of pyrene by different exposure routes and excretion of 1-OHP in urine, in general, 1-OHP urinary excretion levels increase during the course of a workday, reaching maximum values 3–9 h after the end of work (morning time), where post-shift 1-OHP excretion can be lower than pre-shift levels in the case of a worker being exposed occupationally to PAHs on the day prior to sampling (VIAU, 1999).

Other researchers have collected urine samples for 1-OHP analysis immediately after the exposure period (after-work) and early morning. When urine samples were collected after work, bed-time and pre-shift (early morning) (Mcclean *et al.* (2012b), the authors found that bedtime and pre-shift concentrations of urinary 1-OHP were significantly higher than the concentration of 1-OHP in samples collected post-shift. The difference between beginning and post-work excretion will give an indication of the average exposure over the workday, as the environmental exposure to PAHs is associated with increased excretion of 1-OHP (Roth *et al.*, 2001). Urinary 1-OHP in end-of-shift samples ranged from the limit of detection 0.5 (µmol/mol creatinine) to 60 (µmol/mol creatinine) with a mean of 2.49 (µmol/mol creatinine) (Unwin *et al.*, 2006). McClean *et al.* (2004) found that the mean urinary 1-OHP levels among pavers increased significantly from pre-shift to post-shift during each work day.

The consumption of chargrilled food increased the 1-hydroxypyrene and hydroxyphenanthrene concentrations by 24% (95% CI: 11%, 37%) and 17% (95% CI: 8%, 26%), respectively (Alghamdi *et al.*, 2015b). In addition, the urinary levels of naphthalene (U-Nap), phenanthrene (U-Phe), monohydroxylated metabolites of naphthalene (OH-Nap) and phenanthrene (OH-Phe), and 1-hydroxypyrene (OH-Pyr) were significantly associate with dermal uptake measurements in the evening after a given work shift, suggesting that bedtime measurements might be useful for investigating dermal PAH exposures (Sobus *et al.*, 2009). Therefore, it is suggested that the consumption of chargrilled food, dermal uptake, and accumulation of BaP over a working-day might increase 1-OH levels in urine samples in the morning.

5.4.7. Chapter Summary

The chapter discussed the use of urinary 1-OHP levels as exposure biomarkers of BaP in street janitors, and the association between BaP concentration and the urinary 1-OHP levels of the participants in areas associated high and low traffic volumes. The results of the present study indicated that the after work-urinary 1-OHP levels were higher among workers in areas associated high rather than low traffic volumes. Moreover, the morning 1-OHP levels were higher compared to after-work levels, and the possibility of using urinary concentration of 1-OHP as a surrogate biomarker of exposure to airborne BaP was proposed.

Moreover, urinary 1-OHP levels were often high during the holy months and consistently low during normal months. A positive relationship was found between BaP concentration and 1-OHP levels in urine samples.

CHAPTER SIX

Conclusion and Recommendations

Chapter 6: Conclusion and Recommendations

6.1. Conclusion

This research had four objectives: 1) to identify the hot spots of BaP concentrations in Madinah and compare these with background sites; 2) to identify the major variables (i.e. traffic density, religious events, meteorological variations) that could affect BaP concentrations during one Islamic calendar year; 3) to quantify urinary 1-OHP levels as exposure biomarkers of BaP in street janitors; and, 4) to explore the association between BaP concentration and the emergence of urinary 1-OHP biomarker in the urine samples collected from street janitors. From the findings, possible recommendations for the control of BaP in Madinah can thus be made.

To achieve objectives one and two, air pollution with regards to BaP concentration was monitored in five locations: Downtown, Quba, Awali, Sultana and Al-Azizyah. Of these locations, Downtown, Quba and Awali were regarded as high traffic locations as they were located within the city, and hence they received high traffic volume. Sultana and Al-Azizyah were regarded as control locations located on the outskirts of the city, and so they received low traffic volume. Two measurement methods were used to collect and analyze air samples for BaP concentration in these locations: a high-volume air sampler installed at each location and operated for 24 h, and berthing zone air samplers carried by street janitors and operated for 8 h according to their working hours.

The study found that the major hot spots for BaP and other PAH exposure in Madinah are areas associated with high traffic volumes, which according to the findings of the research are Downtown, Quba and Awali. This can be extended to other areas which receive high traffic volumes within Madinah as Downtown, Quba and Awali were selected to represent areas within Madinah that experience high traffic volume. Therefore, any region within Madinah that experiences high traffic volume is a major hot spot for BaP exposure. Given that BaP is a component of motor vehicle exhaust, the higher the traffic, the higher the BaP concentration, and, therefore, areas associated with high traffic volumes are the hot spots of BaP exposure in Madinah city. The research also found that BaP exposure in areas associated with low traffic volumes was relatively low compared to areas associated with high traffic volume. As a result, these regions are not hot spots for BaP exposure, with some of these locations, according to the research findings, including Sultana and Al Aziziyah. In this regard, it can be said that hot spot areas for exposure to BaP in Madinah are areas of religious significance, such as mosques and other places where people gather. Exposure to BaP and other air pollutants is high during the months of religious importance such as Ramadan (M9), Dhu al-Qadah (M11), Dhu al-Ḥijjah (M12) and Muḥarram (M1).

It is, however, important to note that the correlation between BaP and traffic volume in this study tended to be higher compared to those found by previous studies. The reason for this difference is that the selected control locations experience extremely low traffic as they are far from the city centre and not of religious importance.

The research also found that the months in which people are likely to experience high quantities of BaP exposure are months considered to be holy by the Muslim community. These months are Ramadan (M9), Dhu al-Qadah (M11), Dhu al-Ḥijjah (M12) and Muḥarram (M1). During these months, a larger number of Muslim pilgrims from all over the world visit Madinah city, resulting in higher than normal traffic volumes. This increases BaP concentration in the city compared to other months.

In terms of CO concentration, the research found that just, as is with BaP concentration, the holy months in the Islamic calendar, such as Ramadan (M9), Dhu al-Qadah (M11), Dhu al-Ḥijjah (M12) and Muḥarram (M1), are associated with higher CO concentration than the normal months, for the same reason mentioned above. Since CO is a major component present in motor vehicle fumes, the higher the traffic, the higher the CO concentration.

To achieve objectives three and four, morning and after-work urine samples were collected from each of the participants in each of the five locations every day except for the weekends for 12 Islamic months. The urine samples were then analyzed for urinary 1-OHP concentrations. The research found that urine samples collected in locations with high traffic volumes, such as Downtown, Quba and Awali, were associated with higher morning and after-work urinary 1-OHP levels than the control areas of Sultana and Al-Azizyah. The results also indicated that urine samples collected during holy months in the Islamic calendar such as Ramaḍan, Dhu al-Qadah, Dhu al-Ḥijjah and Muḥarram were also associated with high levels of urinary 1-OHP. The main reason for this is that traffic areas and holy months are associated with higher traffic volumes, resulting in higher BaP concentrations than the control areas and normal months. BaP hydrolyzes into 1-OHP once absorbed into the body, and so the higher the exposure to BaP, the higher the concentration of 1-OHP in urine.

A comparison of concentration of morning and after-work 1-OHP levels indicated that morning 1-OHP concentrations were generally higher than the after-work concentration. The reason for this is that 1-OHP has a half-life of 12 to 20 hours, which means it can fully manifest itself in urine 12 to 20 hours after exposure. Given that morning urine samples were collected around 14 hours after exposure while after-work samples were collected around two hours after exposure, the morning samples gave more accurate measurements for BaP exposure. Therefore, it is recommended that, to compile an accurate picture of BaP exposure, urine samples need to be collected in the morning.

The results also found a positive relationship between BaP concentration and 1-OHP concentration in urine samples, such that a high BaP concentration corresponds to a high 1-OHP concentration. This is because, when BaP metabolizes, 1-OHP is a major product of this metabolism, estimated to account for 90% of BaP metabolites. This study thus confirms that urinary 1-OHP levels can function as BaP exposure biomarkers in street janitors.

It is important to note that this is not the first study investigating the relationship between traffic and BaP (or PAH) exposure, as many studies have been carried out before. These studies include: Fan *et al.* (2012b), Kim *et al.* (2014b), Leem *et al.* (2005), and Chuang (2007). However, most of studies these concentrated on traffic police offers, drivers, school children, those working in the fire department, road pavers, mail workers, gardeners, volunteers, and office workers. Little or no attention was given to council workers, especially the city janitors. This is regardless of the fact that most of the work carried out by these people is carried out outdoors, which is heavily polluted with traffic, especially in areas associated with high traffic volume. Because of this they may also be exposed to PAHs. This study bridged a gap by investigating the extent to which city cleaners are exposed to PAHs as they carry out their day to day activities. The findings of the study showed Madinah city janitors, as people in other professions such as taxi drivers, mechanics, drivers, street vendors, workers in mining industry and metal working, and street janitors, especially those working in high traffic volume areas, are exposed to high quantities of BaP, thus increasing their chance of cancer, gene mutations, respiratory complications and reproductive health problems.

6.2. Research Limitations

Because of resource limitations, this research was only carried out in Madinah city, and carbon monoxide concentration was monitored in just one location. As such, the results from this city may not be applicable in other cities in Saudi Arabia, especially cities which do not have religious importance to the Islamic community, and whose meteorological conditions differ from those of Madinah. Also, not comparing CO concentrations with other locations means no comparison can be made of CO concentration between areas associated with high and low traffic volumes.

Moreover, the study did not rely on meteorological monitoring stations fixed in the five locations of the study but in different sites in Madinah city, and therefore it is not possible to make an accurate conclusion about the relationship between the meteorological factors and the concentration of benzopyrene in the surrounding ambient atmosphere.

The study was also concerned with only PAH pollution, especially BaP. However, motor vehicle exhaust is also associated with other several kinds of organic pollutants and inorganic pollutants, such as oxides of nitrogen and sulphur dioxide, which can also affect human health.

It is important to note that accurate estimation of human exposure to air pollutants is extremely important in appraising the risks associated with the pollution, and in designing and implementing strategies that can be used to limit or control these risks (Watson *et al.*, 1988b). Additionally, using ambient measurements to quantify individual exposure may not adequately characterize the individual exposure. This is because people spend different times in different locations, and their activities in these locations depend on gender, occupation, age and socioeconomic status (Watson *et al.*, 1988b). Many occupational estimations are often carried out based on the ambient conditions of air using outdoor fixed site monitors (Watson *et al.*, 1988b); however, basing the measurements on ambient conditions is biased as people spend more time indoors compared to outdoors. Spengler and Sexton (1983) and the National Research Council (1981) have shown that the concentration of air pollutants inside buildings (especially in environments close to the source of pollution) is high compared to the outdoor concentration. This study, however, mainly concentrated on the outdoor PAH pollution by studying occupational individual exposure of street janitors to PAHs and the overall ambient air condition with regard to PAH pollution. Nonetheless, there are individuals who spend most of their time indoors in

Downtown, Quba and Awali, such as shop keepers, school children, company workers and people who live in these areas, especially those who spend most of the time along the highways. These individuals who spend most of the time indoors may be exposed more to PAH when compared to outdoors, owing the fact that once PAHs find their way inside buildings it becomes very difficult to remove them as there is no free flow of wind inside the building. The PAHs may keep on circulating inside the buildings for long periods of time, and so it is suggested that future studies be carried out on the exposure of individuals who spend most of their time indoors in Downtown, Quba and Awali. A comparison may be made with those who spend their time outdoors. This way, a complete understanding on the extent of PAH pollution in Madinah may be understood.

6.3. Recommendations

It is without a doubt that religious activities have the greatest impact on BaP exposure over any other factor. This is because holy months are associated with extremely high traffic volumes, which result in higher BaP concentrations. It is also without doubt that areas associated with high traffic volumes are associated with high BaP exposure. As a result, it is recommended that efforts to reduce the number of vehicles in Madinah be adopted. Two approaches are suggested: enacting laws to reduce the number of cars in high traffic areas, and encouraging the use of hybrid buses, electric trains and cars in Madinah. For enactment of the traffic laws for reducing the number of cars in traffic areas, the following measures are recommended:

i. Enact a law banning the use of older cars in Madinah. Vehicle mileage and age have been associated with emissions. For example, Caserini *et al.* (2013) found that high mileage and older cars tend to produce more pollutants than newer cars. This will, therefore, reduce the emission of polluting gases from exhausts as newer cars pollute less compared to older cars;

ii. Enact laws that prevent heavy vehicles from entering the city centre and the streets leading to them, especially during peak times.

As far as encouraging the use of environmentally friendly means of transport in Madinah is concerned, it is recommended that:

i. The government should offer subsidies, reduce taxes and give tax credits to people importing hybrid and/or electric cars, buses and other electric vehicles;
ii. Light rail electric rails (metros) be built in Madinah. Such rails and trains would help transport people within Madinah without polluting the city.

The research also found that, just like other workers (such as traffic police offers, drivers, school children, those working in fire department, road pavers, mail workers, gardeners, volunteers and office workers), the street janitors in Madinah, especially those located in regions associated with high traffic volume, are also exposed to large quantities of BaP, which is often associated with cancer, gene mutations, respiratory problems and reproductive health problems. As a result, it is recommended that work schedules for the street janitors, especially those working in areas associated with high traffic volumes, be changed to non-rush hours or even night time, when traffic volume is low. This will lower the risk of their exposure to BaP.

6.4. Future Studies

Since this study was carried out only in Madinah, as already discussed, its findings may not be applicable to other cities in Saudi Arabia, especially cities whose weather and climatic conditions are not the same as those of Madinah and/or cities which do not have religious importance to the Muslim world. As result, the above recommendations may not be applicable in other cities. It is, therefore, recommended that similar studies be carried out in other major cities in Saudi Arabia, such as Mecca, Jeddah, Riyadh and Dammam, taking into consideration that the participants are street janitors.

Since this research concentrated on PAH pollution and not inorganic pollutants such as oxides of nitrogen and sulphur dioxide, which can also affect human health, it is recommended that future studies be carried out on exposure to inorganic pollutants in Madinah. This will help the relevant authorities understand the true picture of Madinah's air pollution and the risks that may be associated with it.

Since the destination for most Muslim pilgrims visiting Madinah is the Prophet Muhammad's Mosque (Al Masjid an-Nabawi), the area around the mosque is often associated with high traffic, which makes it one of the potential areas with a high risk of BaP exposure. It is, therefore, recommended that an air pollution study for both organic and inorganic pollutants be carried out in the mosque's parking areas and the surroundings. This will help the relevant authorities to understand the extent of air pollution in the area.

This study has indeed proved that areas associated with high traffic volume and holy months in the Islamic calendar are associated with high BaP concentrations, although the study did not associate the BaP exposure in Madinah with health problems such as cancer and chest problems. However, according to Kim *et al.* (2014a) and Unwin *et al.* (2006), PAHs such as BaP are associated with carcinogenic and mutagenetic properties. It is therefore recommended that an epidemiological study be carried out to associate the rise of air pollution in Medina during religious events and the rate of admission of patients with chest diseases to the emergency department.

Additionally, the study did not make CO comparisons between areas of high and low traffic volume. CO concentration was only monitored in Awali, which is an area associated with high traffic volume, but avoiding a comparison of CO concentrations with other locations does not give a comparison in CO concentration between areas associated with high and low traffic volumes. Therefore, when future studies on CO concentration are carried out, it is recommended that CO concentration be compared between regions of high and low traffic. This way Madinah CO concentration can be associated with traffic volume.

Appendix

7.1. Support letter and ethical approval from Madinah regional municipality

بسم الله الرحمن الرحيم

المملكة العربية السعودية
وزارة الشئون البلدية والقروية
أمانة منطقة المدينة المنورة
الرمز (٢٦٦/٣٠٠)

رقم المعاملة : 12755
تاريخ الخطاب: 16/03/2016
المرفقات :

From: Madinah Municipality

To: Newcastle University

We hereby verify,
The Madinah Regional Municipality is pleased to offer PhD student Khalid Kordi all required cooperation and support to carry out his research in the city of Madinah in terms of harnessing the materials and human resources to serve his research. We will also provide all necessities for monitoring air pollution, collecting samples, and analysis by modern devices available in our laboratories. Furthermore, as for the recruiting of all city cleaners who are participants in his research, the municipality will offer full assistance in obtaining urine samples. All information and data needed for his research will be provided. Please note that all his research expenditure should be deducted from the assigned bench fee account.

Dr. Khalid AbdullQader Taher
Mayor of Madinah Regional Municipality
16/03/2016

هاتف : ٨٢٢٧٤٠٠ - فاكس : ٨٢٢٢٤٩٨ - ص.ب : ٤٩٥٢
بريد الأمانة الالكتروني : webmaster@amana-md.gov.sa موقع الأمانة الالكتروني : www.amana-md.gov.sa

7.2. Support letter from healthy cities program in Madinah

هدفنا .. بيئة صحية متكاملة
بالمدينة المنورة

To whom it May concern .....

We inform you that we are willing to cooperate with the researcher: Khalid Nael Kordi , in all aspects of his research on the subject of air pollution and its impact on public health in Medina.

Also, we do not mind to provide him for all the necessary information and records as he needs such as monitoring of air pollution, health record, traffic data, census population and weather and climate information.

We wish him success in his mission.

Ahmad Obaid Hammad

Coordinator of the Healthy Cities Programmer in Medina

No. : 266/37/HC	Date: 2nd Nov. 2015	Attaches:

المملكة العربية السعودية - المدينة المنورة - مركز طيبة للكشف المبكر - طريق سلطانة - خلف أسواق الدخيل بلازا
ص.ب: 1977 المدينة المنورة 41441 هاتف: 052 51 84 (14) 966+ / 054 51 84 (14) 966+ فاكس: 052 51 84 (14) 966+ تحويلة (106)
info@hcp-med.org www.hcp-med.org

7.3. University's ethical approval

Ethics Form Completed for Project: Traffic Related Air Pollution and Human Health in Madinah – Saudi Arabia

Policy & Information Team, Newcastle University <noreply@limeservice.com>

Wed 16/12/2015 13:03

To: Khalid Kordi (PGR) <K.N.S.Kordi1@newcastle.ac.uk>;

Reference: 2578/2015

Thank you for submitting the ethical approval form for the project 'Traffic Related Air Pollution and Human Health in Madinah – Saudi Arabia'. Expected to run from 28/09/2015 to 28/12/2018.

Based on your answers the University is satisfied that your project has met its ethical expectations and that no further review is required before you begin your research. Please be aware that if you make any significant changes to your project then you should complete this form again as further review may be required. If you have any queries please contact res.policy@ncl.ac.uk

Please quote your reference in any correspondence.

Best wishes

Policy & Information Team, Newcastle University Research Office
res.policy@ncl.ac.uk

7.4. Interview form

Institute of Health&Society

Newcastle University

Institute of Health & Society
Newcastle University
The Baddiley-Clark Building
Richardson Road
Newcastle upon Tyne
NE2 4AX
United Kingdom

Participant's Code	Age	Gender	Height	Weight	Health status	Smoking	Ethnicity	Nationality	Work hours	Time of work	Work area
D013	34	M	169	82	Excellent	Non	Asia	Bengali	8	3-11 pm	downtown
D014	28	M	159	77	Excellent	Non	Asia	Bengali	8	3-11 pm	downtown
D015	26	M	173	86	Excellent	Non	Asia	Bengali	8	3-11 pm	downtown
D016	31	M	156	82	Excellent	Non	Asia	Bengali	8	3-11 pm	downtown
D017	28	M	166	78	Excellent	Non	Asia	Bengali	8	3-11 pm	downtown
D018	34	M	167	75	Excellent	Non	Asia	Bengali	8	3-11 pm	downtown
D019	35	M	168	73	Excellent	Non	Asia	Bengali	8	3-11 pm	downtown
D020	29	M	165	81	Excellent	Non	Asia	Bengali	8	3-11 pm	downtown
Q021	33	M	162	78	Excellent	Non	Asia	Bengali	8	3-11 pm	Quba
Q022	32	M	168	82	Excellent	Non	Asia	Bengali	8	3-11 pm	Quba
Q023	34	M	156	73	Excellent	Non	Asia	Bengali	8	3-11 pm	Quba
Q024	32	M	158	73	Excellent	Non	Asia	Bengali	8	3-11 pm	Quba
Q025	26	M	161	77	Excellent	Non	Asia	Bengali	8	3-11 pm	Quba
Q026	26	M	160	73	Excellent	Non	Asia	Bengali	8	3-11 pm	Quba
Q027	28	M	170	78	Excellent	Non	Asia	Bengali	8	3-11 pm	Quba
Q028	30	M	174	73	Excellent	Non	Asia	Bengali	8	3-11 pm	Quba
Q029	29	M	166	82	Excellent	Non	Asia	Bengali	8	3-11 pm	Quba
Q030	31	M	157	76	Excellent	Non	Asia	Bengali	8	3-11 pm	Quba
Q031	28	M	175	77	Excellent	Non	Asia	Bengali	8	3-11 pm	Quba
Q032	32	M	164	74	Excellent	Non	Asia	Bengali	8	3-11 pm	Quba
Q033	25	M	174	76	Excellent	Non	Asia	Bengali	8	3-11 pm	Quba
Q034	26	M	170	82	Excellent	Non	Asia	Bengali	8	3-11 pm	Quba
Q035	35	M	174	78	Excellent	Non	Asia	Bengali	8	3-11 pm	Quba
Q036	35	M	171	74	Excellent	Non	Asia	Bengali	8	3-11 pm	Quba
Q037	35	M	160	80	Excellent	Non	Asia	Bengali	8	3-11 pm	Quba
Q038	26	M	162	80	Excellent	Non	Asia	Bengali	8	3-11 pm	Quba
Q039	30	M	156	80	Excellent	Non	Asia	Bengali	8	3-11 pm	Quba
Q040	32	M	165	76	Excellent	Non	Asia	Bengali	8	3-11 pm	Quba
A041	31	M	159	74	Excellent	Non	Asia	Bengali	8	3-11 pm	Awali

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Newcastle University

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Richardson Road
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NE2 4AX
United Kingdom

Participant's Code	Age	Gender	Height	Weight	Health status	Smoking	Ethnicity	Nationality	Work hours	Time of work	Work area
A042	32	M	156	81	Excellent	Non	Asia	Bengali	8	3-11 pm	Awali
A043	30	M	168	86	Excellent	Non	Asia	Bengali	8	3-11 pm	Awali
A044	27	M	161	76	Excellent	Non	Asia	Bengali	8	3-11 pm	Awali
A045	30	M	167	77	Excellent	Non	Asia	Bengali	8	3-11 pm	Awali
A046	31	M	162	76	Excellent	Non	Asia	Bengali	8	3-11 pm	Awali
A047	34	M	171	80	Excellent	Non	Asia	Bengali	8	3-11 pm	Awali
A048	29	M	159	75	Excellent	Non	Asia	Bengali	8	3-11 pm	Awali
A049	30	M	168	73	Excellent	Non	Asia	Bengali	8	3-11 pm	Awali
A050	34	M	157	79	Excellent	Non	Asia	Bengali	8	3-11 pm	Awali
A051	32	M	161	77	Excellent	Non	Asia	Bengali	8	3-11 pm	Awali
A052	30	M	175	76	Excellent	Non	Asia	Bengali	8	3-11 pm	Awali
A053	32	M	159	74	Excellent	Non	Asia	Bengali	8	3-11 pm	Awali
A054	29	M	165	80	Excellent	Non	Asia	Bengali	8	3-11 pm	Awali
A055	28	M	175	80	Excellent	Non	Asia	Bengali	8	3-11 pm	Awali
A056	34	M	175	86	Excellent	Non	Asia	Bengali	8	3-11 pm	Awali
A057	33	M	161	77	Excellent	Non	Asia	Bengali	8	3-11 pm	Awali
A058	29	M	164	84	Excellent	Non	Asia	Bengali	8	3-11 pm	Awali
A059	28	M	164	83	Excellent	Non	Asia	Bengali	8	3-11 pm	Awali
A060	33	M	160	79	Excellent	Non	Asia	Bengali	8	3-11 pm	Awali
S061	27	M	168	85	Excellent	Non	Asia	Bengali	8	3-11 pm	Sultana
S062	28	M	157	84	Excellent	Non	Asia	Bengali	8	3-11 pm	Sultana
S063	30	M	174	82	Excellent	Non	Asia	Bengali	8	3-11 pm	Sultana
S064	25	M	175	73	Excellent	Non	Asia	Bengali	8	3-11 pm	Sultana
S065	34	M	156	77	Excellent	Non	Asia	Bengali	8	3-11 pm	Sultana
S066	28	M	172	76	Excellent	Non	Asia	Bengali	8	3-11 pm	Sultana
S067	30	M	169	80	Excellent	Non	Asia	Bengali	8	3-11 pm	Sultana
S068	35	M	168	73	Excellent	Non	Asia	Bengali	8	3-11 pm	Sultana
S069	33	M	162	86	Excellent	Non	Asia	Bengali	8	3-11 pm	Sultana
S070	32	M	170	77	Excellent	Non	Asia	Bengali	8	3-11 pm	Sultana

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Participant's Code	Age	Gender	Height	Weight	Health status	Smoking	Ethnicity	Nationality	Work hours	Time of work	Work area
S071	32	M	159	84	Excellent	Non	Asia	Bengali	8	3-11 pm	Sultana
S072	34	M	161	78	Excellent	Non	Asia	Bengali	8	3-11 pm	Sultana
S073	34	M	163	78	Excellent	Non	Asia	Bengali	8	3-11 pm	Sultana
S074	30	M	167	74	Excellent	Non	Asia	Bengali	8	3-11 pm	Sultana
S075	30	M	177	76	Excellent	Non	Asia	Bengali	8	3-11 pm	Sultana
S076	27	M	172	84	Excellent	Non	Asia	Bengali	8	3-11 pm	Sultana
S077	28	M	176	74	Excellent	Non	Asia	Bengali	8	3-11 pm	Sultana
S078	26	M	177	83	Excellent	Non	Asia	Bengali	8	3-11 pm	Sultana
S079	32	M	178	82	Excellent	Non	Asia	Bengali	8	3-11 pm	Sultana
S080	35	M	166	79	Excellent	Non	Asia	Bengali	8	3-11 pm	Sultana
Z081	32	M	167	77	Excellent	Non	Asia	Bengali	8	3-11 pm	Al Aziziyah
Z082	30	M	165	85	Excellent	Non	Asia	Bengali	8	3-11 pm	Al Aziziyah
Z083	25	M	172	86	Excellent	Non	Asia	Bengali	8	3-11 pm	Al Aziziyah
Z084	33	M	160	79	Excellent	Non	Asia	Bengali	8	3-11 pm	Al Aziziyah
Z085	30	M	166	76	Excellent	Non	Asia	Bengali	8	3-11 pm	Al Aziziyah
Z086	29	M	159	74	Excellent	Non	Asia	Bengali	8	3-11 pm	Al Aziziyah
Z087	25	M	159	81	Excellent	Non	Asia	Bengali	8	3-11 pm	Al Aziziyah
Z088	28	M	158	75	Excellent	Non	Asia	Bengali	8	3-11 pm	Al Aziziyah
Z089	33	M	165	86	Excellent	Non	Asia	Bengali	8	3-11 pm	Al Aziziyah
Z090	35	M	177	73	Excellent	Non	Asia	Bengali	8	3-11 pm	Al Aziziyah
Z091	29	M	167	76	Excellent	Non	Asia	Bengali	8	3-11 pm	Al Aziziyah
Z092	29	M	174	82	Excellent	Non	Asia	Bengali	8	3-11 pm	Al Aziziyah
Z093	32	M	165	85	Excellent	Non	Asia	Bengali	8	3-11 pm	Al Aziziyah
Z094	27	M	157	75	Excellent	Non	Asia	Bengali	8	3-11 pm	Al Aziziyah
Z095	34	M	161	77	Excellent	Non	Asia	Bengali	8	3-11 pm	Al Aziziyah
Z096	25	M	159	82	Excellent	Non	Asia	Bengali	8	3-11 pm	Al Aziziyah
Z097	31	M	163	79	Excellent	Non	Asia	Bengali	8	3-11 pm	Al Aziziyah
Z098	34	M	174	74	Excellent	Non	Asia	Bengali	8	3-11 pm	Al Aziziyah
Z099	35	M	176	84	Excellent	Non	Asia	Bengali	8	3-11 pm	Al Aziziyah
Z100	32	M	166	75	Excellent	Non	Asia	Bengali	8	3-11 pm	Al Aziziyah

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NE2 4AX
United Kingdom

Accommodation

Location: company campus
Type of building: Ground floor lines made by concrete.
Room space: 5m-5m
The number of residents: 4 in each room.
Beds Type: Bunk bed
Heating System: Electrician
Air Cooling System: Air Conditioner
Are there green spaces? Yes
Are there windows in the rooms? Yes
Is there good ventilation? Yes
Is there an indoor kitchen? No
Are there an internal Toilet? No
Are there any polluted sources? No
Are the vehicles allowed to enter into the accommodation? No
Are allowed to use the incense? No
Can they smoke inside their accommodation? No
How far is the motorway from the accommodation? 5ML

Catering

Location: company campus
Is it allowed for workers to cook their own food? No
Is it allowed for workers to have their food from outside? No
How many meals are provided to workers per day? 3
What are the varieties of food per meal?
Breakfast: Eggs-Cheese-Bread-Jam-Butter-Bean-Lentils-Fruits
Lunch: Chicken Gravy-Beef Gravy-Roasted- Fried Chicken -Fish- Rice-Rice with vegetables-Boiled Vegetables-Crisp-Mashed Potato-Pastry-Fruits.
Dinner: Eggs-Cheese-Bread-Bean-Salad-Fruits.
Is there any grill food? No
Is there dining hall: Yes
Has the kitchen any direct access to the dining hall? No

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Transportation

What the distance between the accommodation and work areas? approximately 15 miles

How long is the journey from the accommodation and work areas? approximately 20 miles

What kind of transportation used? Coach

What is the coach capacity? 55

What is the coach model? 2016

What kind of fuel used? Deasil

Is there any traffic density during the round trip? No

Is it allowed for workers to use any other transport to reach their work areas? No

7.5. Participants consent form

بسم الله الرحمن الرحيم

المملكة العربية السعودية
وزارة الشؤون البلدية والقروية
أمانة منطقة المدينة المنورة
الرمز (٢٦٦/٣٠٠)

Consent Form / অনুমতি ফরম

1. I have been informed about the purpose of this study and I have understood the information given to me.
আমি এই গবেষণার উদ্দেশ্য সম্পর্কে অবহিত করা হয়েছে এবং আমি আমার দেওয়া তথ্য বুঝেছি.

2. I voluntarily agree to participate in the project.
আমি স্বেচ্ছায় প্রকল্পে অংশগ্রহণ করতে সম্মত হন.

3. I understand that I can withdraw from the study at any time without giving any reason and that I will not be penalised for withdrawing, nor will I be questioned on why I wish to withdraw.
আমি বুঝতে পারি যে আমি কোন কারণ ছাড়াই যে কোন সময়ে অধ্যয়ন থেকে পৃথক হলে এবং আমি প্রত্যাহার শাস্তি প্রদান করা হবে না, আর আমি কেন আমি প্রত্যাহার করতে ইচ্ছুক জিজ্ঞাসিত হবে.

4. I understand that all responses will be treated in the strictest confidence and any personal details which would reveal my identity will not be published.
আমি বুঝতে পারি যে সমস্ত প্রতিক্রিয়া কঠিন আত্মবিশ্বাস গণ্য করা হবে এবং কোন ব্যক্তিগত বিবরণ যা আমার পরিচয় প্রকাশ হবে প্রকাশ করা হবে না.

5. I understand that the results of this study will be used as part of a PhD thesis at Newcastle University as well as for subsequent publications in academic journals and presentations at academic conferences.
আমি বুঝতে পারি যে এই গবেষণা ফলাফল নিউকাসল বিশ্ববিদ্যালয়ের পিএইচডি থিসিসের অংশ হিসেবে সেইসাথে একাডেমিক সম্মেলন একাডেমিক পত্রিকা ও উপস্থাপনা পরবর্তী প্রকাশনায় জন্য ব্যবহার করা হবে.

Name / নাম:

Date / তারিখ:

Signature/ স্বাক্ষর:

هاتف : ٨٢٢٧٤٠٠ - فاكس : ٨٢٢٢٤٩٨ - ص.ب : ٤٩٥٢
بريد الأمانة الالكتروني : webmaster@amana-md.gov.sa موقع الأمانة الالكتروني : www.amana-md.gov.sa

7.6. Data collection trip

المملكة العربية السعودية
وزارة الشئون البلدية والقروية
أمانة منطقة المدينة المنورة
الرمز (٣٠٠/٢٦٦)

بسم الله الرحمن الرحيم

رقم المعاملة : 12755
تاريخ الخطاب : 26/02/2017
المرفقات :

إلى من يهمه الأمر

اسم الباحث	جهة البحث	موضوع البحث
خالد نائل سليمان كردي	جامعة نيوكاسل	دراسة أثر عوادم السيارات على الصحة العامة

السلام عليكم ورحمة الله وبركاته

نفيدكم بأن الباحث الموضح اسمه وبياناته بعاليه قد أنجز رحلته العلمية لدينا بإدارة البحوث والمختبرات البيئية التابعة لأمانة منطقة المدينة المنورة خلال الفترة من 15 ديسمبر 2016 إلى 19 ديسمبر 2017.

متمنين له دوام التوفيق والنجاح.

وتقبلوا تحياتنا ، ، ،

مدير إدارة البحوث والمختبرات البيئية

فهـــد بن أحمد عبد الله الجـــاسم

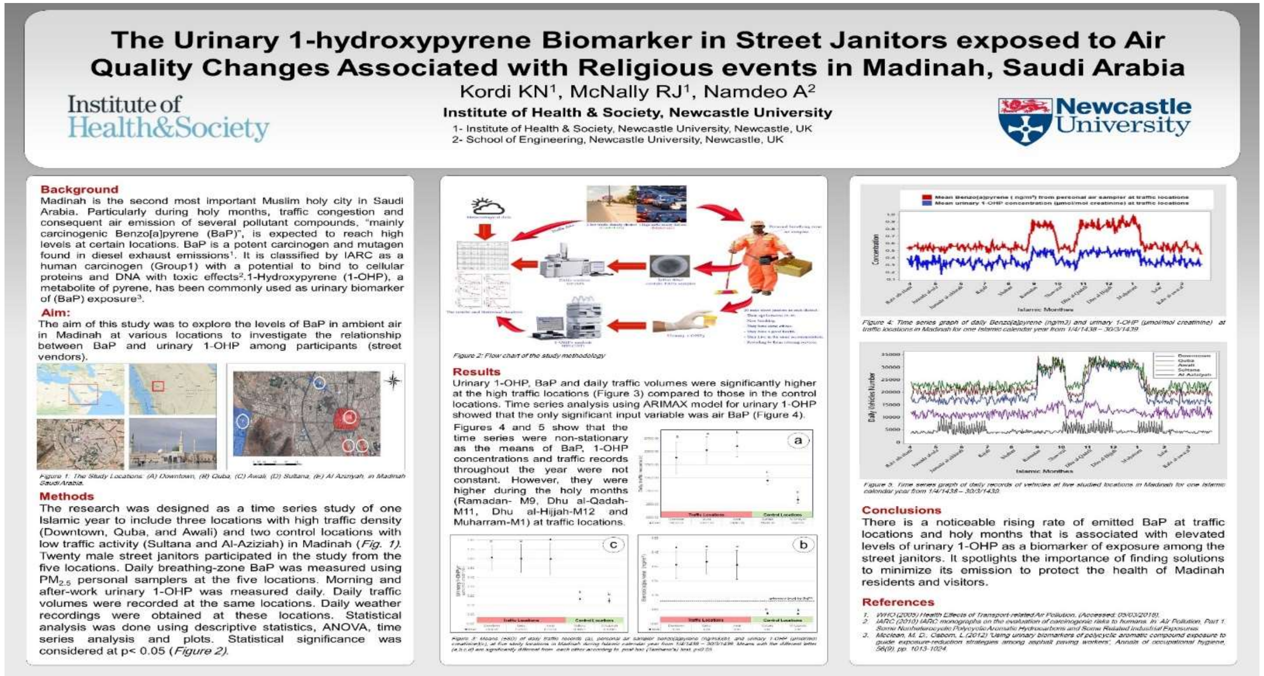
To whom It may concern

We would like to let you know that **Khalid Kordi** has been done his research in Madinah

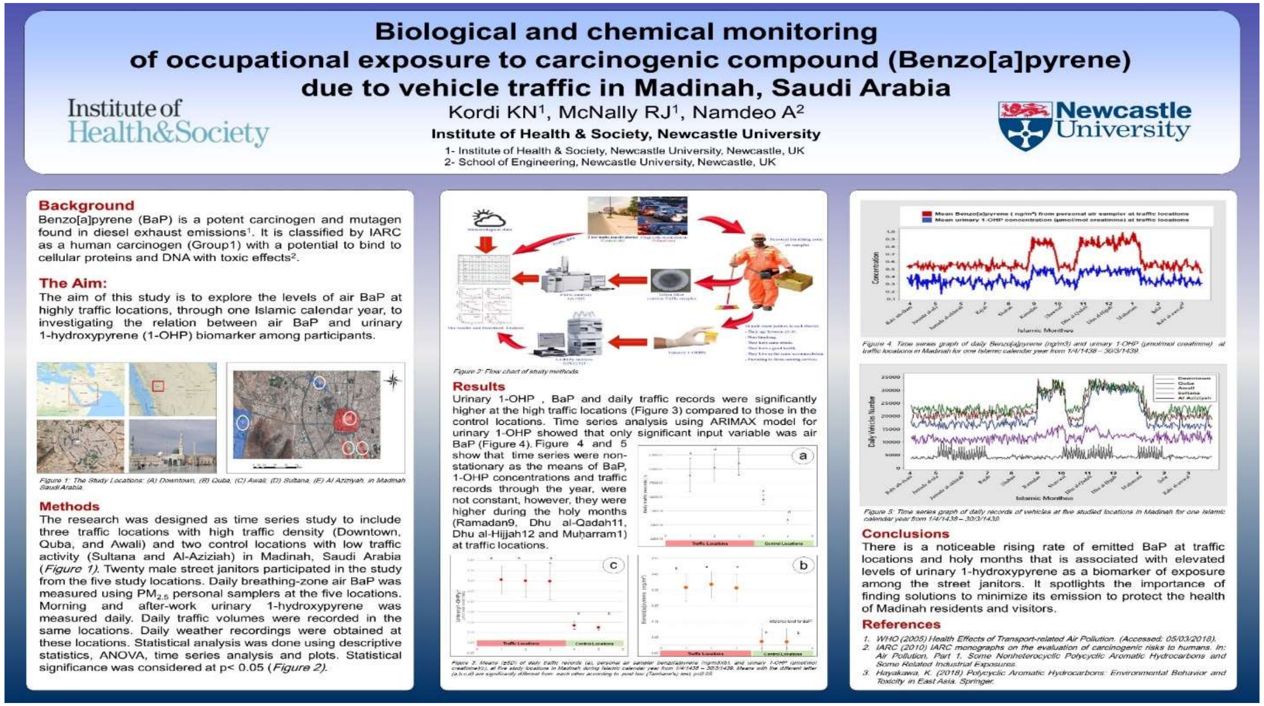
Municipality Laboratories from 15 Dec 2016 to 19 Dec 2017.

هـــاتـــف : ٨٢٢٧٤٠٠ – فـــاكـــس : ٨٢٢٢٤٩٨ – ص.ب : ٤٩٥٢
بريد الأمانة الالكتروني : webmaster@amana-md.gov.sa موقع الأمانة الالكتروني : www.amana-md.gov.sa

7.7. Poster presentation at the 3rd Annual Newcastle University Postgraduate Cancer Conference and International Conference on Transport & Health (ICTH) 24-27 June 2018-Mackinac Island (USA).



7.8. Poster in The IHS Annual PGR, Newcastle University, 5th of June 2018



7.9. Publication in Journal of Transport & Health

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Outline

- Background
- Methods
- Results
- Conclusions

ELSEVIER

Journal of Transport & Health
Volume 9, Supplement, June 2018, Pages S52-S53

2308 - The Urinary 1-Hydroxypyrene Biomarker in Street Janitors Exposed to Air Quality Changes Associated with Religious Events in Madinah, Saudi Arabia

Khalid Kordi [1], Richard McNally [1], Anil Namdeo [2]

⊞ Show more

https://doi.org/10.1016/j.jth.2018.05.038

Background

Madinah is the second most important Muslim holy city in Saudi Arabia. Exceptionally during holy months, traffic congestion and consequent air emission of several pollutant compounds mainly carcinogenic Benzo[a]pyrene (BaP) is expected to reach high levels at certain places. Inhalation of air BaP emissions is the main route of entry into the human body. Therefore, the aim of the present study was to assess a biomarker of exposure to air BaP which is urinary 1-hydroxypyrene (1-OHP) among janitors and find out the relation between this biomarker with daily exposure to air BaP, some traffic and weather factors.

7.10. Replacement of missing values in time series analysis

Some missing values were present in four of the parameters of this study (ambient and individual BaP) due to lack of sampling in off days or weekends (n=114). Because time series analysis requires no missing data, missing values during weekends were replaced by the mean of nearby points.

The span of the nearby points was two valid values above and below the missing value. To check that the replaced values were not far away from real values, a comparison between the values of those four variables and CO concentrations in Awali during the work days and weekends were done as the CO concentration was released parallel with other emissions and traffic volumes and had no missing values. No marked difference was found, confirming that the replaced values were very close to real values.

For confirmation, the binary logistic regression model was used to determine if there were any differences in (CO concentration and the number of vehicles) at Awali during weekend and weekdays. The binary outcome was either weekend or weekday. The explanatory variables were CO and the number of vehicles at Awali. The modelling was carried and ensured that there was no significant difference between the CO levels in weekends and weekdays.

Table 7-1: ***Deviance in ambient CO concentration (ppm) of workdays/weekends and in number of vehicles during the study year.***

Source	DF	Adj. Dev.	Adj. Mean	Chi-Square	P-Value
Regression	2	0.551	0.2755	0.55	0.759
CO	1	0.532	0.5318	0.53	0.466
Vehicles	1	0.450	0.4503	0.45	0.502
Error	351	424.585	1.2096		
Total	353	425.136			

The result of this modelling is shown in Table 7-1. The Chi-squared test ensured that there was a relationship between the two categorical variables tested. The deviance R-squared value was found to be 0.13% and the adjusted deviance R-squared was zero, indicating that changes in the number of vehicles were associated with changes in the CO concentrations.

On the other hand, the negative coefficient corresponding to the number of vehicles as shown in Table 7-2 represented the decrease in odds. Also, the Variance Inflation Factor (VIF) was calculated and found to be small and equal indicating no significant multicollinearity problems between CO concentrations and number of vehicles exist. At 0.05 significance level, the odds ratio for the two variables were ≥ 1, indicating that these two variables are highly correlated. Accordingly, there was no significant variation in the CO values between the weekends and weekdays.

Table 7-2: ***Coefficients and Odds Ratios for the tested variables***

Term	Coefficient	SE Coefficient	VIF	Odds Ratio	95% CI
Constant	-0.662	0.734			
CO	0.341	0.467	2.90	1.4066	(0.5634, 3.5116)
Vehicles	-0.000032	0.000048	2.90	1.0000	(0.9999, 1.0001)

The final regression equation was created based on the sum of the collected data during weekends and weekdays to generate the coefficients of CO and number of vehicles.

$$P(\text{Weekend}) = \exp(Y')/(1 + \exp(Y'))$$

$$Y' = -0.662 + 0.341\ \text{CO} - 0.000032\ \text{Vehicles}$$

According to Hosmer-Lemeshow Test, the probability that a chi-square statistic with df = 8 will be 7.21 or greater is p = 0.514 (Table 7-3) indicating no significant difference between CO concentrations and number of vehicles.

Table 7-3: ***Goodness of Fit Tests***

Test	DF	Chi-Square	P-Value
Deviance	351	424.58	0.004
Pearson	351	354.08	0.444
Hosmer-Lemeshow	8	7.21	0.514

Overall, the emitted CO concentrations were highly correlated with the traffic volume data as illustrated in figure 7-1. The CO concentrations approximately plotted the same pattern during weekdays as weekends.

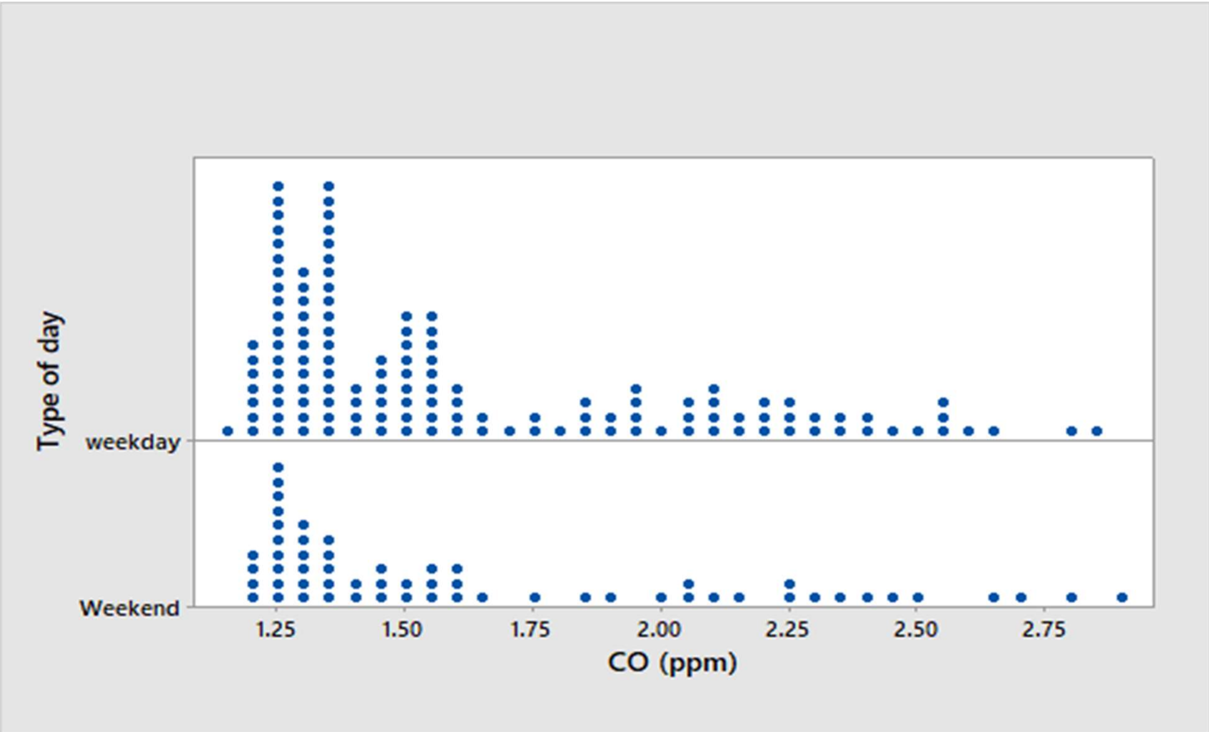
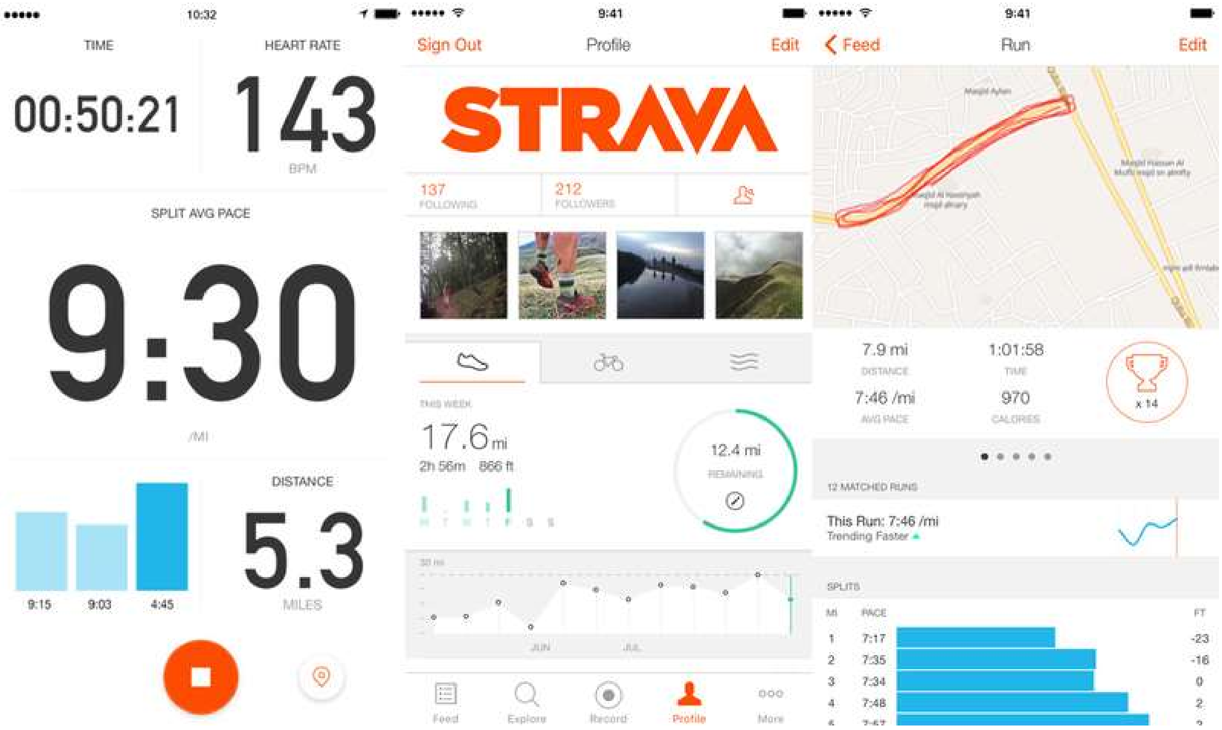


Figure 7-1: Plot of CO concentrations (ppm) against type of days (weekdays & weekends)

7.11. Strava GPS Application



7.12. Health Report Form

وزارة الصحة
Ministry of Health

DETAILED CANDIDATE REPORT

Medical Center Name: Address Phone: Fax: Email: Website URL:	G.C.C CODE NO	
	GCC Slip NO	
	Date Examined:	Report Expiry Date:

CANDIDATE INFORMATION

Name		Age		Maritial Status		PHOTO
Height		Weight	Kgs	Nationality		
Place of issue		Travelling To		Passport No		
Profession		Visa No		Visa Date		
Gender						
GCC Slip No		Date Examined		Report Expiry		

History of any significant past illness including : 1) Allergy: ______ 2) Others: ______ 3) Psychiatric and neurological disorders (Epilepsy, depression, schizophrenia) : ______

'I hereby permit the : ____________Medical Center and the undersigned physician to furnish such information the company may need pertaining to my health status and other pertinent and medical findings and do hereby release them from any and all legal responsibility by doing so. I also certify that my medical history contained above is true and any false statement will disqualify me from my employment, benefits and claims.'

MEDICAL EXAMINATION		LABORATORY INVESTIGATION	
TYPE OF EXAMINATION	RESULTS	TYPE OF LAB INVESTIGATION	RESULTS
EYE	Lt. /6 Rt. /6	**URINE**	
Comment.		SUGAR	
Ear	Lt. Rt.	ALBUMIN	
SYSTEMIC EXAM:		BILHARZIASIS (IF ENDEMIC)	
CARDIOVASCULAR		**STOOL**	
B.P		ROUTINE	
HEART		1. HELMINTHES	
RESPIRATORY		2. GIARDIA	
LUNGS		3. BILHARZIASIS (IF ENDEMIC) CULTURE	
CHEST X-RAY		4. SALMONELLA/SHEGELLA	
GASTRO INTESTINAL: ABDOMEN		5. CHOLERA (IF ENDEMIC)	
OTHERS: HERNIA		**BLOOD**	
VARICOSE VEINS		BLOOD GROUP	
EXTREMITIES		HAEMOGLOBIN	
DEFORMITIES		THICK FILM FOR	
SKIN		1. MALARIA	
VENEREAL DISEASES: CLINICAL		2. MICRO FILARIA	
C.N.S		SEROLOGY	
PSYCHIATRY		1. R.B.S	
Remarks:		2. L.F.T.	
Dear Sir/Madam, Mentioned above is the medical report for Mr./Miss ____________________ who is FIT/UNFIT for the above mentioned job according to the GCC Criteria.		3. CREATININE	
		ELISA	
		1.HIV I & II	
		2. HBs Ag	
		3.Anti HCV	
		VDRL	
		TPHA (IF VDRL POSITIVE)	

BAR CODE

P.O Box 7431 - 11462, Riyadh | Phone : 966 1 4885270 | Fax : 966 1 4885266 | EmailID: info@gcchmc.org

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